



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 33/566, C12N 15/12, A61K 38/19, C07K 14/705	A1	(11) International Publication Number: WO 97/39357 (43) International Publication Date: 23 October 1997 (23.10.97)
(21) International Application Number: PCT/US97/06049 (22) International Filing Date: 11 April 1997 (11.04.97) (30) Priority Data: 60/015,307 12 April 1996 (12.04.96) US (71) Applicants: THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY [US/US]; Stanford, CA 94305 (US). JOHNS HOPKINS UNIVERSITY [US/US]; 725 N. Wolfe Street, PCTB Baltimore, MD 21205 (US). (72) Inventors: BRINK, Marcel; Stanford University School of Medicine, Beckman Center B-271, Stanford, CA 94305 (US). SAMOS, Cindy, H.; 346 Colorado Avenue, Palo Alto, CA 94306 (US). WANG, Yansu; Johns Hopkins School of Medicine, Howard Hughes Medical Institute, 725 N. Wolfe Street, PCTB Baltimore, MD 21205 (US). HSIEH, Jen-Chih; Johns Hopkins School of Medicine, Howard Hughes Medical Institute, 725 N. Wolfe Street, PCTB Baltimore, MD 21205 (US). ANDREW, Deborah; Johns Hopkins School of Medicine, Howard Hughes Medical Institute, 725 N. Wolfe Street, PCTB Baltimore, MD 21205 (US). NATHANS, Jeremy; Johns Hopkins School of Medicine, Howard Hughes Medical Institute, 725 N. Wolfe Street,		PCTB Baltimore, MD 21205 (US). NUSSE, Roel; 69 Peter Coutts Circle, Stanford, CA 94305 (US). BHANOT, Purnima; Johns Hopkins School of Medicine, Howard Hughes Medical Institute, 725 N. Wolfe Street, PCTB Baltimore, MD 21205 (US). (74) Agents: SHOLTZ, Charles, K. et al.; Dehlinger & Associates, P.O. Box 60850, Palo Alto, CA 94306-0850 (US). (81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: Wnt RECEPTOR COMPOSITIONS AND METHODS (57) Abstract <p>Wnt receptor compositions and methods of use are disclosed. In particular, methods using Wnt receptors, such as Dfz2, in screens for compounds which modulate the binding of a Wnt polypeptide to a Wnt receptor.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

WNT RECEPTOR COMPOSITIONS AND METHODS

FIELD OF THE INVENTION

The present invention relates to screening methods employing Wnt receptors.

5

REFERENCES

- Auffray, C., and Rougeon, F., *Eur. J. Biochem.* 107:303-314 (1980).
- Ausubel, F.M., *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, Inc., Media, PA (1988).
- 10 Barbas, C.F., *et al.*, *Proc. Natl. Acad. Sci. USA* 89(10):4457 (1992).
- Bunin, B.A., *et al.*, *Proc. Natl. Acad. Sci. USA* 91:4708 (1994).
- Bunin, B.A. and Ellman, J.A., *J. Am. Chem. Soc.* 114:10997 (1992).
- Chan, S.D.H., *et al.*, *J. Biol. Chem.* 267:25202 (1992).
- Cole, A., *et al.*, *J. Neurochem.* 55:1920-1927 (1990).
- 15 Couso, J.P. and Martinez Arias, A., *Cell* 79:259-272 (1994).
- Dooley, C.T., *et al.*, *Proc. Natl. Acad. Sci. USA* 90(22):10822 (1993a).
- Dooley, C.T., *et al.*, *Life Sci.* 52(18):1509 (1993b).
- Ecker, D.J., *et al.*, *Nuc. Acids Res.* 21(8):1853 (1993).
- Eichler, J., *et al.*, *Biochemistry* 32(41):11035 (1993).
- 20 Eisenberg, L.M., *et al.*, *Dev Biol.* 154(1):73-83 (1992).
- Furka, A., *et al.*, *Int. J. Pept. Protein Res.* 37:487-493 (1991).
- Gennaro, A.R., Ed., REMINGTON'S PHARMACEUTICAL SCIENCES (18th ed., Mack Publishing Co., Easton PA (1990)).
- Gilman, A.G., *et al.*, GOODMAN & GILMAN'S THE PHARMACOLOGICAL BASIS OF
- 25 THERAPEUTICS, 9th Ed., McGraw-Hill, New York, (1995).
- Graba, Y., *et al.*, *Development* 121(1):209-218 (1995).
- Harlow, E., *et al.*, ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press (1988).
- Houghten, R.A., *Proc. Natl. Acad. Sci. USA* 85:5131-5135 (1985).
- 30 Houghten, R.A., *Current Biology* 4:564 (1994).
- Houghten, R.A., *et al.*, *BioTechniques* 4:522-528 (1986).
- Houghten, R.A., *et al.*, *Nature* 354:84-86 (1991).
- Houghten, R.A., *et al.*, *BioTechniques* 13:412-421 (1992).
- Kramer, A., *et al.*, *Pept. Res.* 6(6):314 (1993).
- 35 Lam, K.S., *et al.*, *Nature (London)* 354:82-84 (1991).
- Lam, K.S., *et al.*, *Bioorg. Med. Chem. Lett.* 3:419-424 (1993).

Maniatis, T., *et al.*, in MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, NY (1982).

Mullis, K.B., U.S. Patent No. 4,683,202, issued 28 July 1987.

Mullis, K.B., *et al.*, U.S. Patent No. 4,683,195, issued 28 July 1987.

5 Nüsse, R., and Varmus, H.E., *Cell* 69(7):1073-1087 (1992).

O'Connell, P. and Rosbash, M., *Nuc. Acids Res.* 12:5495-5513 (1984).

Oda, H., *et al.*, *J. Cell Biol.* 121:1133-1140 (1993).

Ohlmayer, M.H., *et al.*, *Proc Nat Acad Sci, USA*, 90(23):10922 (1993).

Pearson, W.R., *Methods in Enzymology* 183:63-98 (1990).

10 Pearson, W.R. and Lipman, D.J., *PNAS* 85:2444-2448 (1988).

Peifer, M., *et al.*, *Development* 120:369-380 (1994).

Pinilla, C., *et al.*, *Biotechniques* 13(6):901 (1992).

Pinilla, C., *et al.*, *Gene* 128(1):71 (1993).

Riggelman, B., *et al.*, *Cell* 63:549-560 (1990).

15 Russell, J., *et al.*, *Development* 115:475-485 (1992).

Sambrook, J., *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL 2 Ed., Vol. 2., Cold Spring Harbor: Cold Spring Harbor Laboratory Press (1989).

Sebestyen, F., *et al.*, *Bioorg. Med. Chem. Lett.* 3:413-418 (1993).

Saffen, D., *et al.*, *Proc Nat Acad Sci, USA*, 85:7795 (1988).

20 Van Leeuwen, F., *et al.*, *Nature* 368:342-344 (1994).

Virgilio, A.A., and Ellman, J.A., *J. Am. Chem. Soc.* 116:11580 (1994).

Yanagawa, S., *et al.*, *Genes & Dev.* 9:1087-1097 (1995).

Zuckermann, R.N., *et al.*, *Int. J. Pept. Protein Res.* 40:498-507 (1992).

25 BACKGROUND OF THE INVENTION

Wnt genes encode secreted proteins involved in cell-to-cell signaling. *Wnt* genes play important growth controlling roles, in particular in the mammary gland, and act as oncogenes in mouse mammary tumors. Little is known about the mechanism of action of *Wnt* products, in part because *Wnt* receptors have until now remained unidentified.

30

SUMMARY OF THE INVENTION

In one aspect, the present invention includes an isolated nucleic acid molecule encoding a *Wnt* receptor polypeptide. In a general embodiment, the *Wnt* receptor polypeptide has an amino acid sequence that is greater than about 90% identical to the

amino acid sequence of a Wnt receptor selected from the group consisting of Dfz1, Dfz2, Rfz1, Rfz2, Hfz3, Hfz4, Hfz5, Mfz3, Mfz4, Mfz5, Mfz6, Mfz7, Mfz8, and Cfz1. In a related embodiment, the Wnt receptor has an amino acid sequence that is more than about 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16. In another related embodiment, the Wnt receptor polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

Examples of nucleic acid molecules encoding Wnt receptor polypeptides are provided herein as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13 and SEQ ID NO:15. Preferred embodiments are human Wnt polynucleotides. An exemplary human Wnt polynucleotide has the sequence presented as SEQ ID NO:9.

The invention further includes fragments of polynucleotides encoding full-length WntR, where the fragments are of sufficient length to hybridize selectively with a Wnt polynucleotide sequence or complement thereof, such as a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13 and SEQ ID NO:15. Such fragments are at least 15, preferably at least about 18, 21 or 24, nucleotides in length.

In another aspect, the invention includes an isolated Wnt receptor polypeptide. In a general embodiment, the polypeptide has an amino acid sequence that is more than about 90% identical to the amino acid sequence of a Wnt receptor selected from the group consisting of Dfz1, Dfz2, Rfz1, Rfz2, Hfz3, Hfz4, Hfz5, Mfz3, Mfz4, Mfz5, Mfz6, Mfz7, Mfz8, and Cfz1. In a related embodiment, the polypeptide has an amino acid sequence that is more than about 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16. In another related embodiment, the polypeptide sequence is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

Preferred embodiments are human Wnt polypeptides. An exemplary human Wnt polypeptide has the sequence presented as SEQ ID NO:10.

The invention further includes peptide fragments derived from a full-length WntR polypeptide, where the fragments contain a region of at least seven, preferably at least ten, consecutive amino acids, and where the region has at least about an 80% identity with the residues of a corresponding region of a polypeptide having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

Also included in the invention are antibodies, both monoclonal and polyclonal, specifically-immunoreactive with Wnt receptor polypeptides. Such antibodies may be produced using standard methods (Harlow).

The invention also includes a method of identifying a compound capable of affecting binding of a Wnt polypeptide to a Wnt receptor polypeptide. The method includes (i) contacting such a Wnt receptor polypeptide with a selected Wnt polypeptide, in the presence and absence of a test compound, (ii) measuring the effect of the test compound on the extent of binding between the Wnt polypeptide and the Wnt receptor polypeptide, and (iii) identifying said compound as effective if its measured effect on the extent of binding is above a threshold level. In a general embodiment, the method includes an additional step (iv) comprising preparing a pharmaceutical preparation of a compound identified as effective to alter binding of a Wnt polypeptide to a WntR polypeptide.

In one embodiment, the threshold is a 2-fold or greater inhibition of binding. In another embodiment, the threshold is a 2-fold or greater potentiation of binding. Examples of suitable Wnt polypeptides include *wingless* (Wg); examples of suitable Wnt receptor polypeptides include Dfz2 (e.g., SEQ ID NO:2).

The test compound may be effective to inhibit binding between the Wnt polypeptide and the Wnt receptor or to displace the Wnt polypeptide from the Wnt receptor polypeptide. In one embodiment, the Wnt receptor polypeptide is expressed on the surface of a cell (e.g., *Drosophila* Schneider 2 (S2) cell) transformed with an expression vector encoding said receptor (e.g., Dfz2).

In another embodiment, the Wnt receptor polypeptide is an N-terminal portion of a full-length Wnt receptor polypeptide, the N-terminal portion including the cysteine-rich amino-terminal domain. In one embodiment, the N-terminal portion is part of a fusion with, e.g., the constant domain of human IgG.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying drawings.

5 **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 shows a sequence comparison of Dfz1 and Dfz2.

Figure 2 shows hydropathy profiles of mammalian and nematode frizzled homologues.

Figure 3 shows a computer-generated image of the expression of DFz2 during
10 Drosophila development evaluated by Northern blot.

Figure 4 is a computer-generated image showing that transfection of DFz2 into S2 cells confers a response to Wg protein.

Figure 5 is a computer-generated image made using confocal immunomicroscopy showing binding of Wg protein to Dfz-2 transfected cells.

15 Figure 6 is a computer-generated image showing the binding of metabolically labeled Wg protein to a Dfz-2/Ig fusion protein.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

20 A polynucleotide sequence or fragment is "derived from" another polynucleotide sequence or fragment when it contains the same sequence of nucleotides as are present in the sequence or fragment from which it is derived. For example, a bacterial plasmid contains an insert "derived from" a selected human gene if the sequence of the polynucleotides in the insert is the same as the sequence of the polynucleotides in the
25 selected human gene.

Similarly, a polypeptide sequence or fragment is "derived from" another polypeptide sequence or fragment when it contains the same sequence of amino acids as are present in the sequence or fragment from which it is derived. A polypeptide "derived from" a nucleic acid is a polypeptide encoded by that nucleic acid. For example, a Wnt receptor
30 polypeptide derived from the human genome (also termed "human Wnt receptor polypeptide" or "hWntR") is a polypeptide encoded by an mRNA (or corresponding cDNA) transcribed from a human Wnt receptor gene.

Percent (%) identity, with respect to two amino acid sequences, refers to the % of residues that are identical in the two sequences when the sequences are optimally aligned

and no penalty is assigned to "gaps". In other words, if a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the % identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the "gap" of the first sequence). Optimal alignment is defined as the alignment giving the highest % identity score. Such alignments can be preformed as described herein using the "GENEWORKS" program. Alternatively, alignments may be performed using the local alignment program LALIGN with a ktup of 1, default parameters and the default PAM. The LALIGN program is found in the FASTA version 1.7 suite of sequence comparison programs (Pearson and Lipman, 1988; Pearson, 1990; program available from William R. Pearson, Department of Biological Chemistry, Box 440, Jordan Hall, Charlottesville, VA).

A full-length Wnt receptor (WntR) polypeptide is defined herein as a polypeptide that is a member of the frizzled protein family, encodes a full-length protein, and has at least about a 90% identity with one or more of the following sequences: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

II. Overview of the Invention

The present invention is based on the discovery of a set of novel members of the vertebrate frizzled family of polarity genes, and on the recognition that the frizzled family of polarity genes encodes the receptors for the Wnt family of proteins. The invention is further enhanced by the recognition that the full-length sequence of each member of the frizzled protein family generally shares a substantially greater degree of homology with the full-length sequences of corresponding frizzled proteins in other species (typically about 80% to >95%) than it does with the full-length sequences of other members of the frizzled protein family in the same species (typically about 30% to 60%). Different members of the frizzled family, however, do contain regions within the coding sequences that have high degrees of homology (up to 90% or more) with one another. This feature, combined with similar sizes and hydrophobicity profiles, facilitates the identification of novel members of the frizzled gene family.

Discoveries described herein enable a number of uses and application of the present invention. These uses and applications are exemplified and discussed in detail below.

III. Identification of Dfz2 as the Wg Receptor

Experiments performed in support of the present invention and described in Examples 1-6, below, indicate that *Drosophila frizzled* gene 2 (Dfz2) is a receptor for *wingless* (Wg). Example 1 details the cloning of Dfz2, the sequence of which is illustrated in Figure 1. Hydrophobicity profiles of additional frizzled family members isolated as part of the present invention are shown in Figure 2. Their sequences are presented in the Sequence Listing. Example 2 describes *in situ* hybridization experiments to determine the pattern of Dfz2 expression. Example 3 describes Northern analyses (Fig. 3) showing that Dfz2 is expressed throughout development.

In Example 4, below, *Drosophila* Schneider 2 (S2) cells were transformed with a Dfz2 expression vector and the effects of the Dfz2 ligand, Wg, were assessed by measuring the levels of *armadillo* (Arm) protein in response to Wg application (Peifer, *et al.*, 1994; Riggleman, *et al.*, 1990; Van Leeuwen, *et al.*, 1994). The results, shown in Figure 4, demonstrate that all four Dfz2-transfected S2 cell lines tested showed increased armadillo signal in response to Wg, whereas no such effect was observed with untransfected S2 cells. These results demonstrate that Dfz2 acts as a signal transducing molecule for Wg, consistent with it being a receptor for Wg.

Further support is provided by immunohistochemical analyses described in Example 5. These experiments were designed to address whether Wg was capable of binding to the Dfz2-transfected cells. Dfz2-transfected and nontransfected cells were exposed to medium containing Wg protein, washed, stained with an anti-Wg antiserum and a labelled secondary antibody, and imaged using a confocal microscope. Exemplary images, shown in Figs 5A-5F, demonstrate that approximately 80% of Dfz2-transfected S2 cells exposed to Wg protein stained brightly (Fig. 5D) whereas Dfz2-transfected cells in the absence of Wg protein (Fig. 5A) as well as non transfected S2 cells (Fig. 5B) did not. The ability of Wg to bind was also tested in human 293 cells, which are heterologous to the Dfz2 protein. As shown in Fig. 5F, about 10-20% of the transfected cells remained positive, similar to the transfection efficiency of 293 cells. Since 293 cells are of human origin, these results indicate that Wg binds to Dfz2 itself, rather than to a molecule whose expression is induced by Dfz2.

The binding of Wg protein to Dfz2 was further confirmed using a fusion protein containing the cysteine-rich amino-terminal domain of Dfz2, linked to the constant domain of human IgG, as described in Example 6. The fusion protein or IgG control was added to conditioned medium from normal S2 cells, or S2 cells producing Wg (HS-wg/S2), which had been metabolically-labeled with [³⁵S] cysteine and methionine.

The fusion proteins and possible complexes were then isolated and analyzed by gel electrophoresis and fluorography (Fig. 6). Two bands of approximately 52 kd (the size of Wg) were detected in the lane with the Dfz2-Ig fusion added to the medium of HS-wg/S2 cells.

- 5 The above results taken together, particularly the observations that (i) Wg binds to DFz2, and (ii) the binding leads to a biological response, strongly support the role of Dfz2 as the receptor for the Wg protein.

IV. Novel Frizzled Family Members Identified in Vertebrates

- 10 Experiments performed in support of the present invention have further resulted in the identification of at least six novel frizzled family members in human and mouse. This brings the total number of frizzled-like sequences identified in mammalian genomes to 8, since two (Rfz1 and Rfz2) were previously cloned from rat (Chan, *et al.*, 1992). The six novel genes include Mfz3, Mfz4, Mfz6, Mfz7, and Mfz8, as well as human sequences
- 15 Hfz3, Hfz5 and Hfz7. A sequence 95% identical over 143 amino acids to Hfz5 was PCR-amplified (Mullis, 1987; Mullis, *et al.*, 1987) from mouse genomic DNA using Hfz5-specific primers, suggesting that an Mfz5 gene exists as well. The DNA and translated amino acid sequences of these 6 family members are provided in the Sequence Listing, along with the sequence of a novel family member isolated from *C. elegans* (Cfz1). The
- 20 hydrophobicity profiles of these sequences are presented in Figure 2. These profiles, along with the sequences of regions that are conserved among different frizzled family members, are used in determining whether a polypeptide sequence is a member of the frizzled gene family. According to the present invention, member of this family are considered to be Wnt receptors.

- 25 Using the guidance herein, one of skill in the art can isolate additional members of the frizzled gene family. In particular, probes homologous to regions conserved among the various family members can be designed and used to probe cDNA or genomic DNA libraries. Alternatively or in addition, PCR primers corresponding to such conserved regions may be designed and used to isolate additional sequences from any suitable source
- 30 of DNA, including libraries and reverse transcription (RT) -generated cDNA samples.

V. Wnt Genes and Proteins

Wg in *Drosophila* is part of larger gene family (Eisenberg, *et al.*, 1992; Graba, *et al.*, 1995; Russell, *et al.*, 1992) of Wnt genes. At least 3 homologous genes have been

identified in *Drosophila*, and over 10 Wnt genes have been identified in most vertebrates (Nusse and Varmus, 1992). According to the present invention, the products of these genes are the ligands for receptors encoded by the large family of fz-like genes in vertebrates. Determination of which Wnt gene products are specific to which Wnt receptor may be performed by one of skill in the art following the teachings of the present specification.

All members of the *Wnt* family encode secreted proteins that act as cell-cell signaling molecules. *Wnt* genes play an important role in the control of cell growth, particularly in the mammary gland, and can act as oncogenes in mouse mammary tumors. The proteins contain a signal sequence, one or several N-linked glycosylation sites and many cysteine residues. The product of the mouse *Wnt-1* gene has been studied most extensively. If *Wnt-1* is overexpressed in various cell lines, the protein enters the secretory pathway. The protein can be detected in protease resistant structures, presumably secretory vesicles, and contains carbohydrate structures at several N-linked glycosylation sites. It is thus generally assumed that the *Wnt-1* protein is secreted from cells, although extracellular forms of the protein have been difficult to detect. In addition, most of the intracellular *Wnt-1* protein made in transfected cells is incompletely glycosylated (it remains sensitive to endoglycosidase H) and has probably not traversed the Golgi apparatus. Moreover, much of the *Wnt-1* protein becomes associated with the resident ER protein BiP, indicating that it is incorrectly folded.

In spite of these difficulties, it has been shown that *Wnt-1* overproduction leads to secretion of modest amounts of extracellular protein. The secreted forms have undergone more extensive glycosylations, and may bind to the cell surface or to the extracellular matrix.

VI. Role of Wnt in Cancer

Members of the *Wnt* gene family are important regulators of mammary cell growth. Indeed, *Wnt* genes owe their discovery to their role as oncogenes in mouse mammary cancer: previous experiments which examined the sequence around integration sites for Mouse Mammary Tumor Virus (MMTV) DNA showed that many tumors had sustained proviral insertions near the *Wnt-1* gene, the first member of this gene family. A biological assay for *Wnt-1* was subsequently established using gene transfer experiments. This assay was used to show that certain mammary gland-derived cell lines can be morphologically transformed by *Wnt-1*. Direct evidence that *Wnt-1* expression gives a strong growth stimulus to mammary cells came from transgenic mice carrying *Wnt-1* linked to the MMTV

promoter, which developed mammary hyperplasia and tumors. By infecting primary mammary cells with retroviruses expressing *Wnt-1* and re-implantation of the infected cells, similar hyperplasia of the mammary gland were obtained. Additional experiments led to the identification of a *Wnt-1* related oncogene activated by MMTV insertion, called *Wnt-3*.

5 The growth stimulus generated by the expression of *Wnt-1* in the mammary gland implies that mammary cells are equipped with a Wnt receptor that becomes activated by the *Wnt-1* protein, as well as the other signaling components. While neither *Wnt-1* nor *Wnt-3* are expressed in the normal mammary gland, at least 5 other *Wnt* genes are expressed during specific stages of mammary gland development, including during the rapid expansion
10 of the pre-lactating gland or when the gland regresses.

The oncogenic action of *Wnt-1* and *Wnt-3* is best explained by their acting as ligands for Wnt receptors meant for other *Wnt* genes, and activating these receptors inappropriately. Alternatively, *Wnt-1* and *Wnt-3* may not activate these receptors but may interfere with a ligand-receptor interaction normally leading to regression of the gland.

15 The strong growth stimulus by oncogenic *Wnt* genes and the dynamic expression patterns of other *Wnt* genes in the mammary gland provide evidence that *Wnt* genes are important regulators of mammary gland growth. It is also possible that *WNT* genes other than *WNT-1* and *WNT-3* are involved in human breast cancer. In analogy with the mouse, it is likely that some of these are expressed during the normal cycles of growth of the
20 mammary gland. In contrast to silent genes, genes that are expressed are candidates to become amplified, since the ensuing overexpression of those genes can give a selective advantage to cells even during the first rounds of amplification.

By way of illustration, a survey of mouse mammary tumors identified one tumor where the mouse *Wnt-2* gene was amplified and overexpressed whereas *Wnt-2* had a low
25 level of expression in the normal gland. Further, there was no evidence for insertion of MMTV near *Wnt-2* in that tumor. This finding shows that *Wnt* genes are not necessarily activated only by MMTV, a relevant factor for human breast cancer since that disease has no viral etiology but is often characterized by gene amplification.

30 VII. Screening Methods

In view of the role of Wnt in cancer and other processes involving growth, development and proliferation (both normal and abnormal), it would be desirable to identify modulators of Wnt activity that affect the interactions of specific Wnt proteins with their receptors. Such modulators may, for example, inhibit the binding of Wnt to its receptor

(*e.g.*, by competitive or noncompetitive inhibition), or they may potentiate or stabilize the binding. The recognition that members of the frizzled family of proteins can act as receptors for the Wnt family of proteins enables a number of screening approaches to the isolation of such modulatory compounds that have heretofore not been possible.

5 Examples of such screening approaches include protein-protein binding assays in which the level of binding of Wnt to its receptor, or a biological consequence of such binding, is measured. The latter assay is exemplified in Example 4, where cells not normally expressing Wnt receptors are transformed with a Wnt receptor (in this case, Dfz2), and the effects of Wnt (in this case, Wg) on the cells are measured (in this case, by
10 detecting levels of Arm). Such cells may be transformed with the Wnt receptor of choice (*e.g.*, any of fz1, fz2, fz3, fz4, fz5, fz6, fz7 or fz8 receptors).

 In Example 4, expression of Arm was detected using a Western blot method. Other methods may be employed which are more suitable for high throughput screening applications. For example, labelled anti-Arm antibodies may be used to directly visualize
15 levels of Arm in multi-well format screen.

 Alternatively, the assays may simply detect the degree of binding between Wnt ligands and Wnt receptors, and not the biological consequences of such binding. For example, cells expressing a selected Wnt receptor may be plated in the wells of a 96-well plate and contacted with a solution containing reporter-labeled Wnt (*e.g.*, radiolabelled or
20 fluorescently-tagged) in the presence and absence of a test compound (*i.e.*, a putative modulator of Wnt/receptor interactions). The effect of the test compound on the extent of binding between Wnt and Wnt receptor is measured, and the compound is identified as effective if its effect on the extent of binding is above a threshold level (*e.g.*, a several-fold difference in binding level between control and experimental samples) In one embodiment,
25 the threshold is a 2-fold difference. In another embodiment, it is a 5-fold difference. In yet another it is a 10-fold or greater difference. The difference in binding in the presence and absence of an effective test compound is preferably statistically-significant, as determined by a standard statistical test.

 It will be appreciated that the putative modulator compound can alternatively be
30 added after the cells had been incubated with labelled Wnt. In a screen for inhibitors of binding, the system is assayed for a decrease in the signal reflecting bound labelled Wnt, or an increase in the signal reflecting labelled Wnt in solution.

 Such a screen may also be employed to screen for potentiators of Wnt/receptor interactions. For example, test compounds may be added to the wells (either during or

after incubation with labelled Wnt), and the wells then contacted with unlabeled Wnt. Test compounds in wells where the unlabelled Wnt is less effective at displacing the bound labelled Wnt are selected for more detailed examination of ability to potentiate Wnt/receptor binding.

- 5 Assays such as described above may also be used to determine the relationship between different Wnt proteins and different receptors. For example, the ligand concentration dependence of binding may be used in measurement of the relative affinities of selected Wnt receptors with selected ligands, and ligands with a selected affinity for the receptor can be examined further using, *e.g.*, *in vitro* or *in vivo* assays. In this manner,
10 one of skill in the art can identify which Wnt protein(s) is optimally paired with which receptor(s).

- In cases where the Wnt ligand has been matched to a specific Wnt receptor (*e.g.*, in the case of Wg and Dfz2), the receptor/ligand pair can be used in, *e.g.*, screening applications. For example, the pair may be used in a binding assay to screen for
15 compounds which are effective to modulate the binding of the specific ligand to its receptor. These methods enable the identification of compounds with two general types of activities: (i) those which act generally, *e.g.*, on a class of Wnt/Wnt receptor pairs, to disrupt or facilitate binding, and (ii) those which act selectively disrupt or facilitate the binding between a selected Wnt ligand and its receptor, but not between other Wnt ligands and their
20 receptors.

- Compounds identified by one of the screens described herein may be further evaluated for efficacy using an *in vitro* assay such as described above. Further, such compounds may be tested in *in vivo* models employing Wnt/Wnt receptor interactions. For example, the compounds may be tested in a mouse mammary tumor model for effectiveness
25 at inhibiting growth of mammary tumors.

VIII. Compounds Suitable for Screening

- A variety of different compounds may be screened using methods of the present invention. They include peptides, macromolecules, small molecules, chemical and/or
30 biological mixtures, and fungal, bacterial, or algal extracts. Such compounds, or molecules, may be either biological, synthetic organic, or even inorganic compounds, and may be obtained from a number of sources, including pharmaceutical companies and specialty suppliers of libraries (*e.g.*, combinatorial libraries) of compounds.

In cases where an identified active compound is a peptide, the peptide may be utilized to design a peptoid mimetic and aid in the discovery of orally-active small molecule mimetics. Alternatively, the peptides themselves may be used as therapeutics.

Further, the structure of a bioactive polypeptide may be determined using, for example, NMR, and may be used to select the types of small molecules screened.

Methods of the present invention are well suited for screening libraries of compounds in multi-well plates (*e.g.*, 96-well plates), with a different test compound in each well. In particular, the methods may be employed with combinatorial libraries. A variety of combinatorial libraries of random-sequence oligonucleotides, polypeptides, or synthetic oligomers have been proposed (Kramer, *et al.*, 1993; Houghten, 1985, 1994; Houghten, *et al.*, 1986, 1991, 1992; Ohlmayer, *et al.*, 1993; Dooley, *et al.*, 1993a-1993b; Eichler, *et al.*, 1993; Pinilla, *et al.*, 1992, 1993; Ecker, *et al.*, 1993; and Barbas, *et al.*, 1992). A number of small-molecule libraries have also been developed (*e.g.*, Bunin, *et al.*, 1994; Bunin and Ellman, 1992; Virgilio and Ellman, 1994).

Combinatorial libraries of oligomers may be formed by a variety of solution-phase or solid-phase methods in which mixtures of different subunits are added stepwise to growing oligomers or parent compound, until a desired oligomer size is reached (typically hexapeptide or heptapeptide). A library of increasing complexity can be formed in this manner, for example, by pooling multiple choices of reagents with each additional subunit step (Houghten, *et al.*, 1991).

Alternatively, the library may be formed by solid-phase synthetic methods in which beads containing different-sequence oligomers that form the library are alternately mixed and separated, with one of a selected number of subunits being added to each group of separated beads at each step (Furka, *et al.*, 1991; Lam, *et al.*, 1991, 1993; Zuckermann, *et al.*, 1992; Sebestyen, *et al.*, 1993).

The identity of library compounds with desired effects on the binding of a Wnt to a Wnt receptor can be determined by conventional means, such as iterative synthesis methods in which sublibraries containing known residues in one subunit position only are identified as containing active compounds.

30

IX. Pharmaceutical Preparations of Active Compounds

After identifying certain test compounds as potential WntR agonists or antagonists, the practitioner of the screening assay will typically continue to test the efficacy and specificity of the selected compounds both *in vitro* and *in vivo*. Whether for subsequent *in*

vivo testing, or for administration to an animal as an approved drug, agents identified in the screening assay can be formulated in pharmaceutical preparations for *in vivo* administration to an animal, preferably a human.

The compounds selected in the screening assay, or a pharmaceutically acceptable salt thereof, may accordingly be formulated for administration with a biologically acceptable medium, such as water, buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like) or suitable mixtures thereof. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to medicinal chemists. As used herein, "biologically acceptable medium" includes any and all solvents, dispersion media, and the like which may be appropriate for the desired route of administration of the pharmaceutical preparation. The use of such media for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the activity of the compound, its use in the pharmaceutical preparation of the invention is contemplated.

Suitable vehicles and their formulation inclusive of other proteins are described, for example, in Gennaro, 1990. These vehicles include injectable "deposit formulations". Based on the above, such pharmaceutical formulations include, although not exclusively, solutions or freeze-dried powders of the compound in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered media at a suitable pH and isosmotic with physiological fluids. In a preferred embodiment, the compound can be disposed in a sterile preparation for topical and/or systemic administration. In the case of freeze-dried preparations, supporting excipients such as, but not exclusively, mannitol or glycine may be used and appropriate buffered solutions of the desired volume will be provided so as to obtain adequate isotonic buffered solutions of the desired pH. Similar solutions may also be used for the pharmaceutical compositions in isotonic solutions of the desired volume and include, but not exclusively, the use of buffered saline solutions with phosphate or citrate at suitable concentrations so as to obtain at all times isotonic pharmaceutical preparations of the desired pH (for example, neutral pH).

The following examples illustrate but in no way are intended to limit the present invention.

MATERIALS AND METHODS

Unless otherwise indicated, restriction enzymes and DNA modifying enzymes were obtained from New England Biolabs (Beverly, MA) or Boehringer Mannheim (Indianapolis, IN). Nitrocellulose paper was obtained from Schleicher and Schuell (Keene, NH). Other
5 chemicals were purchased from Sigma (St. Louis, MO) or United States Biochemical (Cleveland, OH). Unless otherwise specified, the experiments were performed using standard methods (Ausubel, *et al.*, 1988; Sambrook, *et al.*, 1989; Harlow, *et al.*, 1988).

A. Buffers

Phosphate-buffered saline (PBS)

10x stock solution, 1 liter:

80 g NaCl

2 g KCl

11.5 g Na₂HPO₄·7H₂O

2 g KH₂PO₄

Working solution, pH 7.3:

137 mM NaCl

2.7 mM KCl

4.3 mM Na₂HPO₄·7H₂O

1.4 mM KH₂PO₄

EXAMPLE 1

Molecular Cloning of Dfz2

25 Polymerase chain reaction (PCR; Mullis, 1987; Mullis, *et al.*, 1987) primer pools YW157 and YW158 were designed based on sequences (SEQ ID NO:16, SEQ ID NO:17, respectively) conserved in Dfz1, Human frizzled 3 (Hfz3), Rat frizzled 1 (Rfz1) and Rat frizzled 2 (Rfz2). The primer pools were completely degenerate, that is, each possible codon of each amino acid in SEQ ID NO:16 and SEQ ID NO:17 was represented in the
30 respective primer pool, with the exception that the wobble base of the 3'-most codon was not included in YW157. The primers were used to amplify *Drosophila* genomic DNA, resulting in an amplification product that, when sequenced, was found to contain a novel *frizzled* family member - Dfz2. The PCR product was used to isolate genomic clones of Dfz2 from an adult *Drosophila* genomic library (Maniatis, *et al.*) and cDNA clones from a
35 0-24 hr cDNA library.

The amino acid sequence of Dfz2 was compared to that of Dfz1 by aligning the sequences as shown in Fig. 1. Dfz2 and Dfz1 are 32% identical. Identical residues are

indicated in the consensus and the conserved cysteine residues in the cysteine-rich domain are in bold-face. The sequence alignments were done using the "GENEWORKS" program.

Hydropathy values were calculated using the "MACVECTOR" 3.5 software according to the Kyte-Doolittle software and a window size of 15 amino acids.

5

EXAMPLE 2

In Situ RNA Hybridization

In situ hybridization experiments were performed to determine the pattern of Dfz2 expression. Freshly dissected adult brains, whole embryos or heads were rapidly frozen in plastic molds placed on a dry ice/alcohol slurry and processed for sectioning as described previously (Cole, *et al.*, 1990). ³⁵S-Labeled antisense riboprobes were prepared from linearized p"BLUESCRIPT" plasmid subclones using either T3 or T7 RNA polymerase. In situ hybridization was performed as described by Saffen, *et al.*, and hybridized sections were exposed to X-ray film and digitized.

15

EXAMPLE 3

Expression of DFz2 During *Drosophila* Development

The expression pattern of DFz2 was assessed using Northern (RNA) blot analysis. Total RNA was isolated using the LiCl-Urea precipitation method (Auffray and Rougeon, 1980). 30 microgram of RNA from each sample was resolved on a formaldehyde 1% agarose gel. The RNA was transferred to a nylon filter, cross-linked by UV irradiation and hybridized to a probe made by random priming Dfz2 or RP49 DNA fragments using standard methods (Sambrook, *et al.*, 1989). In other experiments, Poly (A)⁺ RNA from various stages of *Drosophila* development was first selected from total RNA using the Invitrogen "FASTTRACK" 2.0 kit and 5 µg was loaded per lane.

Exemplary results are shown in Figure 3. A 4.0 kb transcript was detected in embryonic stages 0-2; 2-3; 4-5; 9-12, first, second and third instar larvae and pupae. A transcript of similar size was observed in *Drosophila* clone-8 cells (cl-8), a cell line from imaginal discs previously shown to be responsive to Wg activity *in vitro*. *Drosophila* Schneider 2 (S2) cells, which do not respond to Wg, did not contain detectable DFz2 transcripts. The blot was also probed for expression of the ribosomal protein RP49 (O'Connell and Rosbash, 1994, lower panel) as a control for RNA integrity and loading.

30

EXAMPLE 4Transfection of Dfz2 in S2 Cells Confers a Response to Wg protein

S2 cells were evaluated for Dfz2 expression because the cells are known not to respond to Wg (Yanagawa, *et al.*, 1995). Since, as described above, the native cells did not express Dfz2, they were used in Dfz2 transfection experiments to determine whether expression of Dfz2 would confer sensitivity to Wg.

An expression vector containing Dfz2 coding sequences under the control of a metal-inducible metallothionein promoter was used to transfect S2 cells using standard methods. Stable cell lines were derived by selection in hygromycin and tested for Dfz2 expression. In cells grown in the absence of inducers, a baseline level of expression was detected with an antiserum to Dfz2. Induction of the metallothionein promoter resulted in increased levels of expression.

Sensitivity of the Dfz2-transfected S2 cells to Wg protein was assessed by measuring the levels of armadillo (Arm) protein in response to Wg application. In intact *Drosophila* embryos and in clone-8 cells, Arm protein migrates in two different forms, differing from each other in phosphorylation. When these cells are incubated in the presence of soluble Wg protein, the level of the faster migrating (non-phosphorylated) form increases (Peifer, *et al.*, 1994; Riggelman, *et al.*, 1990; Van Leeuwen, *et al.*, 1994). This increase can be detected using a standard Western blot assay as described below.

Conditioned medium containing Wg protein was produced by subjecting S2H5wg cells to heat-shock for 30 minutes at 37°C, allowing the cells to recover for 30 minutes at 25°C, and resuspending them in S2 medium without fetal calf serum (FCS). The cells were incubated for 3 hrs to allow secretion of proteins into the medium, after which they were removed by centrifugation (10 min., 2000 xg and 1hr, 100,000 xg, respectively). The conditioned media were concentrated 12-fold ("CENTRIPREP30", Amicon) and used in the experiments as follows.

Clone 8, untransformed S2, and Dfz-transformed S2 (S2Dfz2) cells were incubated for 2 hrs in 6-well dishes in either normal concentrated medium or in concentrated medium from S2 cells producing Wg.

Overexpression of the Dfz2 gene (under control of the metallothionein promoter) was induced by culturing S2Dfz2 and S2 control cells in S2 medium containing 0.5 mM CuSO₄ for 5 hrs prior to the incubation with the conditioned media.

The target cells were lysed in lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet-P40, 5 mM EDTA) supplemented with 20 µg leupeptin, 100 µg aprotinin and

180 μ g PMSF per ml. The extracts were subjected to electrophoresis and Western blotting. Blots were stained in Ponceau Red to evaluate equal loading of total protein and transfer, and then incubated overnight in blocking buffer with monoclonal anti-arm antibody 7A1 at a 1:1000 dilution or rat-polyclonal anti- α -catenin antibody DCAT-1 (Oda, *et al.*, 1993),
5 diluted 1:1000. The blots were washed three times for 15 min each in TBST and incubated for 1 hr with horseradish peroxidase conjugated secondary antibodies (Biorad) diluted 1:20,000 in blocking buffer.

Incubation of Dfz2-transfected S2 cells (but not untransfected S2 cells) in the presence of soluble Wg protein resulted in an increase in the level of Arm protein similar to
10 that observed in *Drosophila* embryos and clone-8 cells. Exemplary results are shown in Fig. 4. Addition of Wg (wingless) results in increased signal intensity of the armadillo band. No such effect is observed with untransfected S2 cells. However, all four independent Dfz2-transfected S2 cell lines, derived from two separate transfections, showed increased armadillo signal in response to Wg (two of the four are shown). Further
15 induction of Dfz2 expression by copper sulphate in the transfected cells led to a slight decrease in the response to Wg. As a control for equal loading, the blots were stripped and incubated with an antiserum against α -catenin (lower panel).

EXAMPLE 5

Wg Protein Binds to Dfz2 Transfected Cells

The results described in Example 4 showed that Dfz2 acts as a signal transducing molecule for Wg, suggesting that it is a receptor for Wg. Immunohistochemical analyses were performed to determine whether Wg was capable of binding to the Dfz2-transfected cells.

25 Nontransfected Sneider 2 (S2) cells and S2 cells expressing Dfz2 were washed twice in PBS and incubated with 1.5 ml of medium alone or 1.5 ml of a 10x concentrated stock of Wg conditioned medium at 4°C for 3 hours. After three 10 minute washes with PBS, the cells were fixed in 2% methanol-free formaldehyde (Polysciences, Inc) for 15 minutes at room temperature. Following three more 10 minute washes with PBS, affinity purified Wg
30 antibody at 1/25 and 5% donkey serum were added to the cells in PBS and incubated overnight at 4°C.

The antiserum was affinity-purified using a bacterial fusion protein containing a domain unique to Wg (the Wg insert -- an 85 amino acid sequence not found in any wg orthologs). Previous experiments have indicated that this domain is dispensable for Wg

activity, that it probably does not participate in the interactions between Wg and its receptor.

Following 3 additional 10 minute washes, fluorescent-labeled cy3 secondary antibody, donkey anti-rabbit (Sigma), at 1/100 and 5% donkey serum were added to the
5 cells for 1 hour at room temperature. The cells were then washed 3 more times in PBS and mounted in Vectashield mounting medium (Vector).

Confocal images were collected with a Bio-Rad MRC 1000 confocal laser attached to a Zeiss Axio scope microscope. Exemplary images are shown in Figs 5A-5F. Normal and transfected cells were incubated with either normal S2 medium (Fig. 5A) or
10 concentrated conditioned medium from S2 cells producing Wg (Figs. 5B, 5C, 5D, 5E, 5F). Dfz2-transfected S2 cells stained brightly in approximately 80% of the cells when incubated with Wg and the antiserum (Figure 5D) whereas Dfz2-transfected cells in the absence of Wg protein (Fig. 5A) as well as non transfected S2 cells (Fig. 5B) showed only some spots of background staining. The positive staining was not uniform over the cell surface but
15 punctate and may reflect clustering of receptor complexes.

The ability of Wg to bind was also tested in heterologous cells (human 293 cells) transiently-transfected with Dfz2. In view of high background binding observed in initial experiments, the transiently-transfected 293 cells were preincubated with chlorate, which inhibits sulfation of proteins and glucosaminoglycans, and with heparatinase, to remove
20 heparin-like molecules. This pre-treatment significantly lowered the background binding (presumably due to Wg binding to extracellular matrix; Fig. 5E). As shown in Fig. 5F, about 10-20% of the transfected cells remained positive, similar to the transfection efficiency of 293 cells. Since 293 cells are of human origin, these results strongly suggest that Wg binds to Dfz2 itself, rather than to a molecule whose expression is induced by
25 Dfz2.

In contrast to the positive staining patterns observed with Dfz2-transfected cells, no staining was detected in S2 cells expressing Notch (Fig. 5C). Notch is a protein that has been previously proposed to act as a receptor for Wg (Couso and Arias, 1994).

The above results taken together indicate that Wg protein can specifically bind to
30 cells expressing Dfz2, and that this binding is not likely due to clonal variation.

EXAMPLE 6Binding of Metabolically-Labeled Wg Protein to a Dfz-2/IgG Fusion Protein

5 The binding of Wg protein to Dfz2 itself was also assayed using a fusion protein containing the cysteine-rich amino-terminal domain of Dfz2, linked to the constant domain of human IgG. The fusion protein or IgG control was added to conditioned medium from normal S2 cells, or S2 cells producing Wg (HS-wg/S2), which had been metabolically-labeled with [³⁵S] cysteine and methionine.

10 The fusion proteins and possible complexes were then retrieved by adding sepharose-ProteinA beads and analyzed by gel electrophoresis and fluorography. Figure 6 shows that the Dfz2 fusion protein, but not the control IgG, selectively binds to labeled proteins of 52 kD, the size of the mature Wg protein. Normal S2 cells did not produce Dfz-2 binding proteins.

15 While the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: The Board of Trustees of the Leland Stanford Junior University, et al.
- (ii) TITLE OF INVENTION: Wnt Receptor Compositions and Methods
- (iii) NUMBER OF SEQUENCES: 18
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Dehlinger & Associates
 - (B) STREET: 350 Cambridge Avenue, Suite 250
 - (C) CITY: Palo Alto
 - (D) STATE: CA
 - (E) COUNTRY: USA
 - (F) ZIP: 94306
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: 11-APR-1997
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/015,307
 - (B) FILING DATE: 12-APR-1996
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Sholtz, Charles K.
 - (B) REGISTRATION NUMBER: 38,615
 - (C) REFERENCE/DOCKET NUMBER: 8600-0167.41
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (415) 324-0880
 - (B) TELEFAX: (415) 324-0960

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2344 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: Dfz2 Polynucleotide, coding region begins at nucleotide #225
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCGCTGTGTC TGAAGGAAAC ACTACCCGCT TTTCCGGCTC TCGAGGCGCC TCCACGAAGG

AGTGAGGTGC	AACCGCAGAG	AAGGTCAGCA	AAGAAAGAGC	AAGGGGTTC	AAGTCACACA	120
ACCGAACTAA	GCTAAGACGC	ACAAAATGAG	ACACAATCGA	CTGAAGGTCC	TGATCCTGGG	180
ACTCGTCCTC	CTGCTGACAT	CTTGTCGAGC	GGATGGACCG	CTGCACAGTG	CGGATCACGG	240
CATGGGCGGA	ATGGGCATGG	GTGGTCACGG	CCTGGACGCG	AGTCCCGCAC	CCGTTTACGG	300
AGTGCCAGCC	ATACCCAAGG	ATCCCAATCT	GCGATGCGAG	GAGATCACCA	TACCAATGTG	360
TCGGGGCATT	GGCTACAACA	TGACATCCTT	CCCCAACGAA	ATGAACCATG	AGACCCAGGA	420
CGAAGCGGGC	CTGGAGGTGC	ACCAGTTCTG	GCCCCTGGTG	GAGATCAAAT	GCTCGCCGGA	480
CCTCAAGTTC	TTCCTGTGCA	GCATGTACAC	GCCCATCTGC	CTGGAGGATT	ACCACAAGCC	540
GCTGCCCCGTT	TGCCGGAGTG	TCTGCGAGAG	AGCCCGCTCG	GGATGCGCAC	CCATCATGCA	600
GCAGTACAGC	TTCGAATGGC	CGGAGAGAAT	GGCGTGCGAG	CACTTGCCCC	TTCATGGTGA	660
CCCCGACAAT	CTGTGCATGG	AACAGCCCCTC	GTACACGGAG	GCTGGCAGCG	GTGGCAGCTC	720
GGGCGGATCG	GGTGGCTCTG	GCAGCGGTTT	CGGCTCCGGC	GGCAAACGGA	AGCAAGGAGG	780
CAGTGGCTCG	GGCGGCAGTG	GGGCCGGCGG	CAGCAGCGGT	TCCACCTCAA	CGAAGCCGTG	840
CCGCGGACGC	AATTCAAAAA	ACTGCCAAAA	TCCCCAAGGA	GAAAAGGCAA	GCGGAAAAGA	900
GTGCAGCTGC	TCGTGCCGCT	CCCCACTCAT	CTTCCTGGGG	AAGGAGCACT	GGCTGCAGCA	960
GCAGTCGCAG	ATGCCCATGA	TGCACCATCC	ACACCACTGG	TACATGAACC	TCACTGTCCA	1020
AAGGATCGCC	GGCGTTCCAA	ACTGCGGCAT	ACCGTGCAAG	GGGCCCTTCT	TCAGCAACGA	1080
CGAAAAGGAT	TTCCGCCGCC	TCTGGATCGC	CCTGTGGTCG	GGACTGTGCT	TCTGCAGCAC	1140
GCTCATGACC	CTAACCACAT	TCATCATCGA	CACCGAAAGG	TTTAAGTACC	CGGAGCGGCC	1200
ATTGTCTTCC	TCTCCGCCTG	CTACTTCATG	GTGGCAGTGG	GCTACCTGTC	GCGCAACTTC	1260
CTGCAGAACG	AGGAGATCGC	CTGCGACGGC	CTGCTGCTCC	GGGAAAGCTC	CACGGGTCCG	1320
CACTCTTGCA	CCCTGGTCTT	CCTGCTCACC	TACTTCTTTG	GCATGGCCTC	GTCCATCTGG	1380
TGGGTGATCC	TCACTTTTAC	CTGGTTCCTG	GCCGCTGGTC	TGAAGTGGGG	CAATGAGGCC	1440
ATCACCAAGC	ACTCGCAGTA	CTTCCATCTG	GCCGCCTGGT	TGATTCCCAC	TGTCCAGTCC	1500
GTGGCCGTAC	TCCTGCTCTC	GGCGGTGGAT	GGCGATCCCA	TTCTGGGCAT	CTGCTATGTG	1560
GGCAACCTCA	ATCCGGATCA	CCTAAAGACC	TTTGTGCTGG	CCCCGCTCTT	AGTTTACCTC	1620
GTAATCGGCA	CCACCTTCCT	GATGGCCGGC	TTTGTGTCCC	TCTTCCGCAT	CCGCTCGGTT	1680
ATCAAGCAAC	AGGGCGGTGT	AGGAGCTGGT	GTCAAGGCGG	ACAAGCTGGA	GAAACTGATG	1740
ATCAGGATTG	GCATCTTCTC	GGTGCTCTAC	ACGGTGCCGG	CCACCATAGT	TATCGGATGT	1800
TACCTGTACG	AAGCAGCCTA	CTTTGAGGAC	TGGATCAAGG	CCCTGGCCTG	TCCATGCGCC	1860
CAGGTGAAGG	GTCCCGGCAA	GAAGCCTCTC	TACTCGGTCC	TGATGCTCAA	GTACTTCATG	1920
GCCCTGGCCG	TGGGCATCAC	CTCGGGCGTG	TGGATCTGGT	CTGGCAAGAC	GCTGGAGAGC	1980
TGGCGACGCT	TCTGGCGGAG	ACTCCTAGGA	GCGCCGGACC	GCACGGGCGC	CAACCAGCTG	2040
GCGATCAAGC	AGCGGCCTCC	GATCCCGCAT	CCCTATGCCG	GATCTGGAAT	GGGCATGCCC	2100

GTGGGCTCGG CGGCGGGCTC CCTGCTGGCC ACGCCCTACA CCCAGGCGGG CGGACGTCGG 2160
 TGGCCTCCAC CAGCCACCAC CACCTGCACC ACCACGTTCT CAAGCAGCCG GCGGCCAGCC 2220
 ACGTATGACA TGGAGAGTCG GGGGGAGCAT CGACCATGGG CGGCGGTGGG GGC GGCGGTA 2280
 CAGCCCTTGG CGGCGGCACC CTGGGCCACG GCACCGCGAT GAGCAGCAGC ACGGTCGGCA 2340
 TGGG 2344

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 694 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: Dfz2 Polypeptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
- | | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Arg | His | Asn | Arg | Leu | Lys | Val | Leu | Ile | Leu | Gly | Leu | Val | Leu | Leu | 1 | 5 | 10 | 15 |
| Leu | Thr | Ser | Cys | Arg | Ala | Asp | Gly | Pro | Leu | His | Ser | Ala | Asp | His | Gly | 20 | 25 | 30 | |
| Met | Gly | Gly | Met | Gly | Met | Gly | Gly | His | Gly | Leu | Asp | Ala | Ser | Pro | Ala | 35 | 40 | 45 | |
| Pro | Gly | Tyr | Gly | Val | Pro | Ala | Ile | Pro | Lys | Asp | Pro | Asn | Leu | Arg | Cys | 50 | 55 | 60 | |
| Glu | Glu | Ile | Thr | Ile | Pro | Met | Cys | Arg | Gly | Ile | Gly | Tyr | Asn | Met | Thr | 65 | 70 | 75 | 80 |
| Ser | Phe | Pro | Asn | Glu | Met | Asn | His | Glu | Thr | Gln | Asp | Glu | Ala | Gly | Leu | 85 | 90 | 95 | |
| Glu | Val | His | Gln | Phe | Trp | Pro | Leu | Val | Glu | Ile | Lys | Cys | Ser | Pro | Asp | 100 | 105 | 110 | |
| Leu | Lys | Phe | Phe | Leu | Cys | Ser | Met | Tyr | Thr | Pro | Ile | Cys | Leu | Glu | Asp | 115 | 120 | 125 | |
| Tyr | His | Lys | Pro | Leu | Pro | Val | Cys | Arg | Ser | Val | Cys | Glu | Arg | Ala | Arg | 130 | 135 | 140 | |
| Ser | Gly | Cys | Ala | Pro | Ile | Met | Gln | Gln | Tyr | Ser | Phe | Glu | Trp | Pro | Glu | 145 | 150 | 155 | 160 |
| Arg | Met | Ala | Cys | Glu | His | Leu | Pro | Leu | His | Gly | Asp | Pro | Asp | Asn | Leu | 165 | 170 | 175 | |
| Cys | Met | Glu | Gln | Pro | Ser | Tyr | Thr | Glu | Ala | Gly | Ser | Gly | Gly | Ser | Ser | 180 | 185 | 190 | |
| Gly | Gly | Ser | Gly | Gly | Ser | Gly | Ser | Gly | Ser | Gly | Ser | Gly | Gly | Lys | Arg | | | | |

195					200					205					
Lys	Gln	Gly	Gly	Ser	Gly	Ser	Gly	Gly	Ser	Gly	Ala	Gly	Gly	Ser	Ser
210					215					220					
Gly	Ser	Thr	Ser	Thr	Lys	Pro	Cys	Arg	Gly	Arg	Asn	Ser	Lys	Asn	Cys
225					230					235					240
Gln	Asn	Pro	Gln	Gly	Glu	Lys	Ala	Ser	Gly	Lys	Glu	Cys	Ser	Cys	Ser
				245					250					255	
Cys	Arg	Ser	Pro	Leu	Ile	Phe	Leu	Gly	Lys	Glu	Gln	Leu	Leu	Gln	Gln
			260					265					270		
Gln	Ser	Gln	Met	Pro	Met	Met	His	His	Pro	His	His	Trp	Tyr	Met	Asn
		275					280					285			
Leu	Thr	Val	Gln	Arg	Ile	Ala	Gly	Val	Pro	Asn	Cys	Gly	Ile	Pro	Cys
					295						300				
Lys	Gly	Pro	Phe	Phe	Ser	Asn	Asp	Glu	Lys	Asp	Phe	Ala	Gly	Leu	Trp
305					310					315					320
Ile	Ala	Leu	Trp	Ser	Gly	Leu	Cys	Phe	Cys	Ser	Thr	Leu	Met	Thr	Leu
				325					330					335	
Thr	Thr	Phe	Ile	Ile	Asp	Thr	Glu	Arg	Phe	Lys	Xaa	Pro	Gly	Ala	Ala
			340					345					350		
Ile	Val	Phe	Leu	Ser	Ala	Cys	Tyr	Phe	Met	Val	Ala	Val	Gly	Tyr	Leu
		355					360					365			
Ser	Arg	Asn	Phe	Leu	Gln	Asn	Glu	Glu	Ile	Ala	Cys	Asp	Gly	Leu	Leu
	370					375					380				
Leu	Arg	Glu	Ser	Ser	Thr	Gly	Pro	His	Ser	Cys	Thr	Leu	Val	Phe	Leu
385					390					395					400
Leu	Thr	Tyr	Phe	Phe	Gly	Met	Ala	Ser	Ser	Ile	Trp	Trp	Val	Ile	Leu
				405					410					415	
Thr	Phe	Thr	Trp	Phe	Leu	Ala	Ala	Gly	Leu	Lys	Trp	Gly	Asn	Glu	Ala
			420					425					430		
Ile	Thr	Lys	His	Ser	Gln	Tyr	Phe	His	Leu	Ala	Ala	Trp	Leu	Ile	Pro
		435					440					445			
Thr	Val	Gln	Ser	Val	Ala	Val	Leu	Leu	Leu	Ser	Ala	Val	Asp	Gly	Asp
	450					455					460				
Pro	Ile	Leu	Gly	Ile	Cys	Tyr	Val	Gly	Asn	Leu	Asn	Pro	Asp	His	Leu
465					470					475					480
Lys	Thr	Phe	Val	Leu	Ala	Pro	Leu	Phe	Val	Tyr	Leu	Val	Ile	Gly	Thr
				485					490					495	
Thr	Phe	Leu	Met	Ala	Gly	Phe	Val	Ser	Leu	Phe	Arg	Ile	Arg	Ser	Val
			500					505					510		
Ile	Lys	Gln	Gln	Gly	Gly	Val	Gly	Ala	Gly	Val	Lys	Ala	Asp	Lys	Leu
		515					520					525			
Glu	Lys	Leu	Met	Ile	Arg	Ile	Gly	Ile	Phe	Ser	Val	Leu	Tyr	Thr	Val
	530					535					540				
Pro	Ala	Thr	Ile	Val	Ile	Gly	Cys	Tyr	Leu	Tyr	Glu	Ala	Ala	Tyr	Phe
545					550					555					560

[illegible]

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2624 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: Mus musculus frizzled-3 protein,
Coding Region: 313..2313

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCGGCA	CGAGAAGATG	GAATCTGTGA	TTTGGGAATG	CGGTTGATGG	AGTTGCTATG	60
CTGGCCAGAT	GTGCCCAATG	TAATAAAATG	AAAAGAAGAT	ACAAGATGAT	GTCATCTTCC	120
CATATTGTGA	AACCAAAAAC	AAATGCCCTT	TGTGAGACCA	GGTTACCAGT	TCTTTGACAG	180
TACAGGGAGT	TTTTAAACTG	AGGAGCCTAA	CAGATAAGGG	GTACTTTCAA	GCTGAGACCT	240
GCAGGCATAT	ACTGATCTAA	AACGCATCTT	GTGTAGATCT	GATCATCCGA	GCCTCATCTT	300
GATCCAGGAA	GAATGGCTGT	GAGCTGGATT	GTCTTTGATC	TTTGGCTCTT	GACTGTGTTT	360
CTGGGGCAGA	TAGGTGGGCA	CAGTTTGTTT	TCTTGTTAAC	CTATAACCTT	GAGGATGTGC	420
CAAGATTTGC	CTTACAATAC	TACCTTCATG	CCTAATCTTC	TGAACCATTA	TGACCAACAG	480
ACTGCAGCTT	TAGCAATGGA	GCCCTTCCAC	CCTATGGTGA	ACCTGGATTG	TTCTCGGGAT	540
TTTCGGCCAT	TTCTTTGTGC	ACTCTATGCC	CCTATTTGTA	TGGAATATGG	ACGTGTCACA	600

CTTCCCTGCC	GTAGGCTGTG	TCAGCGTGCC	TATAGCGAGT	GTTCAAACT	CATGGAGATG	660
TTTGGTGTCC	CGTGGCCTGA	AGATATGGAG	TGCAGTAGGT	TTCCAGATTG	TGATGAGCCA	720
TATCCCCGAC	TTGTGGATTT	GAATTTAGTT	GGAGATCCAA	CTGAAGGAGC	CCCAGTTGCA	780
GTGCAGAGGG	ACTATGGTTT	TTGGTGTCCC	AGAGAGTTAA	AAATTGATCC	TGATCTTGGC	840
TATTCCTTTC	TGCACGTGCG	AGATTGTTTCG	CCACCATGTC	CCAATATGTA	CTTCAGGAGA	900
GAAGAACTGT	CATTTGCTCG	CTATTTTCATA	GGCCTGATTT	CAATCATTTG	CCTCTCTGCC	960
ACATTGTTTA	CTTTTTTAAC	CTTTCCTAATT	GACGTCACAA	GATTCCGTTA	CCCTGAAAGA	1020
CCTATCATAT	TTTATGCAGT	CTGCTACATG	ATGGTGTGTCAT	TAATTTTCTT	CATTGGGTTT	1080
TTGCTGGAGG	ACCGAGTAGC	CTGCAATGCA	TCTAGCCCTG	CACAGTATAA	GGCTTCTACA	1140
GTGACACAAG	GATCTCACAA	TAAGGCCTGT	ACCATGCTCT	TTATGGTACT	ATATTTTTTC	1200
ACTATGGCTG	GCAGTGTATG	GTGGGTAATT	CTTACCATCA	CATGGTTTTT	AGCAGCTGTG	1260
CCAAAGTGGG	GCAGTGAAGC	TATTGAGAAG	AAAGCATTGC	TGTTTCATGC	CAGTGCCTGG	1320
GGCATCCCCG	GAACCTCTAAC	TATCATCCTT	TTAGCGATGA	ATAAAATTGA	AGGTGACAAT	1380
ATTAGTGGCG	TGTGTTTTGT	CGGCCTCTAC	GACGTTGATG	CATTAAGATA	TTTCGTTCTC	1440
GCTCCCCCTCT	GCCTGTATGT	GGTAGTTGGG	GTTTCTCTCC	TTTTAGCCGG	CATTATATCC	1500
CTAAACAGAG	TTCGGATTGA	GATCCCATTA	GAAAAGGAAA	ACCAAGATAA	GTTAGTGAAG	1560
TTCATGATCC	GGATTGGTGT	TTTCAGCATT	CTCTACCTTG	TGCCACTCTT	GGTTGTAATT	1620
GGATGTTACT	TTTATGAGCA	AGCTTACCGC	GGCATCTGGG	AGACAACATG	GATCCAGGAA	1680
CGCTGCAGAG	AGTATCACAT	TCCATGTCCG	TACCAGGTTA	CTCAGATGAG	TCGTCCAGAC	1740
CTGATTCTCT	TTCTGATGAA	GTATCTCATG	GCTCTCATAG	TTGGGATTCC	CTCTATATTT	1800
TGGGTTGGAA	GCAAAAAGAC	ATGCTTTGAA	TGGGCCAGTT	TTTTCCATGG	GCGTAGGAAA	1860
AAAGAGATAG	TGAATGAGAG	CCGGCAGGTG	CTCCAGGAAC	CTGACTTTGC	TCAGTCACTC	1920
CTGAGGGACC	CAAATACTCC	AATTATAAGA	AAATCAAGAG	GAACCTCCAC	TCAAGGGACA	1980
TCCACACATG	CTTCTTCAAC	TCAGCTGGCC	ATGGTGGATG	ACCAAAGAAG	CAAAGCAGGG	2040
AGTGTCCACA	GCAAAGTGAG	CAGCTACCAT	GGCAGCCTCC	ACAGGTCACG	GGATGGCAGG	2100
TACACTCCCT	GCAGTTACCG	AGGAATGGAG	GAGAGACTAC	CTCACGGCAG	CATGTCACGG	2160
CTGACGGATC	ATTCCAGGCA	CAGTAGTTCT	CATCGGCTCA	ACGAGCAGTC	CCGACACAGC	2220
AGCATCCGAG	ACCTCAGTAA	CAACCCCATG	ACTCACATTA	CACATGGCAC	CAGCATGAAC	2280
CGTGTTATTG	AGGAGGATGG	AACCAGTGCT	TAGTCTTGTC	TAAGGTGAAA	TGTGTGCTGT	2340
TGAAAAGCAG	GTTTTGCCTT	CGCATGGCTG	GCTGCTGTAA	CTCACTGTCTG	CTCTGCTTTC	2400
TTGGGCAGAG	TGTCAGCCTG	GGAAAGTAGA	TCTTTGCTCT	TTGTATCACA	TCAACCCTGG	2460
GGTGTGAACA	CATCCAAACC	CTAAGGATCA	TGTCATCACA	AAAGTAATTC	TTTCTAGGCT	2520
GTGAAGAGAT	GATTGTCTGG	TGAGCATTTT	TTATAAACAT	GCTTATTTTA	TATCTAGAAA	2580
AATCCTCTAT	GTGTGGTGAC	TGCTTTGTAG	TGAATTTTAT	ATAA		2624

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 667 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: Mfz3 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Val	Ser	Trp	Ile	Val	Phe	Asp	Leu	Trp	Leu	Leu	Thr	Val	Phe	1	5	10	15
Leu	Gly	Gln	Ile	Gly	Gly	His	Ser	Leu	Phe	Ser	Cys	Glu	Pro	Ile	Thr	20	25	30	
Leu	Arg	Met	Cys	Gln	Asp	Leu	Pro	Tyr	Asn	Thr	Thr	Phe	Met	Pro	Asn	35	40	45	
Leu	Leu	Asn	His	Tyr	Asp	Gln	Gln	Thr	Ala	Ala	Leu	Ala	Met	Glu	Pro	50	55	60	
Phe	His	Pro	Met	Val	Asn	Leu	Asp	Cys	Ser	Arg	Asp	Phe	Arg	Pro	Phe	65	70	75	80
Leu	Cys	Ala	Leu	Tyr	Ala	Pro	Ile	Cys	Met	Glu	Tyr	Gly	Arg	Val	Thr	85	90	95	
Leu	Pro	Cys	Arg	Arg	Leu	Cys	Gln	Arg	Ala	Tyr	Ser	Glu	Cys	Ser	Lys	100	105	110	
Leu	Met	Glu	Met	Phe	Gly	Val	Pro	Trp	Pro	Glu	Asp	Met	Glu	Cys	Ser	115	120	125	
Arg	Phe	Pro	Asp	Cys	Asp	Glu	Pro	Tyr	Pro	Arg	Leu	Val	Asp	Leu	Asn	130	135	140	
Leu	Val	Gly	Asp	Pro	Thr	Glu	Gly	Ala	Pro	Val	Ala	Val	Gln	Arg	Asp	145	150	155	160
Tyr	Gly	Phe	Trp	Cys	Pro	Arg	Glu	Leu	Lys	Ile	Asp	Pro	Asp	Leu	Gly	165	170	175	
Tyr	Ser	Phe	Leu	His	Val	Arg	Asp	Cys	Ser	Pro	Pro	Cys	Pro	Asn	Met	180	185	190	
Tyr	Phe	Arg	Arg	Glu	Glu	Leu	Ser	Phe	Ala	Arg	Tyr	Phe	Ile	Gly	Leu	195	200	205	
Ile	Ser	Ile	Ile	Cys	Leu	Ser	Ala	Thr	Leu	Phe	Thr	Phe	Leu	Thr	Phe	210	215	220	
Leu	Ile	Asp	Val	Thr	Arg	Phe	Arg	Tyr	Pro	Glu	Arg	Pro	Ile	Ile	Phe	225	230	235	240
Tyr	Ala	Val	Cys	Tyr	Met	Met	Val	Ser	Leu	Ile	Phe	Phe	Ile	Gly	Phe	245	250	255	

28

Leu Leu Glu Asp Arg Val Ala Cys Asn Ala Ser Ser Pro Ala Gln Tyr
 260 265 270
 Lys Ala Ser Thr Val Thr Gln Gly Ser His Asn Lys Ala Cys Thr Met
 275 280 285
 Leu Phe Met Val Leu Tyr Phe Phe Thr Met Ala Gly Ser Val Trp Trp
 290 295 300
 Val Ile Leu Thr Ile Thr Trp Phe Leu Ala Ala Val Pro Lys Trp Gly
 305 310 315 320
 Ser Glu Ala Ile Glu Lys Lys Ala Leu Leu Phe His Ala Ser Ala Trp
 325 330 335
 Gly Ile Pro Gly Thr Leu Thr Ile Ile Leu Leu Ala Met Asn Lys Ile
 340 345 350
 Glu Gly Asp Asn Ile Ser Gly Val Cys Phe Val Gly Leu Tyr Asp Val
 355 360 365
 Asp Ala Leu Arg Tyr Phe Val Leu Ala Pro Leu Cys Leu Tyr Val Val
 370 375 380
 Val Gly Val Ser Leu Leu Leu Ala Gly Ile Ile Ser Leu Asn Arg Val
 385 390 395 400
 Arg Ile Glu Ile Pro Leu Glu Lys Glu Asn Gln Asp Lys Leu Val Lys
 405 410 415
 Phe Met Ile Arg Ile Gly Val Phe Ser Ile Leu Tyr Leu Val Pro Leu
 420 425 430
 Leu Val Val Ile Gly Cys Tyr Phe Tyr Glu Gln Ala Tyr Arg Gly Ile
 435 440 445
 Trp Glu Thr Thr Trp Ile Gln Glu Arg Cys Arg Glu Tyr His Ile Pro
 450 455 460
 Cys Pro Tyr Gln Val Thr Gln Met Ser Arg Pro Asp Leu Ile Leu Phe
 465 470 475 480
 Leu Met Lys Tyr Leu Met Ala Leu Ile Val Gly Ile Pro Ser Ile Phe
 485 490 495
 Trp Val Gly Ser Lys Lys Thr Cys Phe Glu Trp Ala Ser Phe Phe His
 500 505 510
 Gly Arg Arg Lys Lys Glu Ile Val Asn Glu Ser Arg Gln Val Leu Gln
 515 520 525
 Glu Pro Asp Phe Ala Gln Ser Leu Leu Arg Asp Pro Asn Thr Pro Ile
 530 535 540
 Ile Arg Lys Ser Arg Gly Thr Ser Thr Gln Gly Thr Ser Thr His Ala
 545 550 555 560
 Ser Ser Thr Gln Leu Ala Met Val Asp Asp Gln Arg Ser Lys Ala Gly
 565 570 575
 Ser Val His Ser Lys Val Ser Ser Tyr His Gly Ser Leu His Arg Ser
 580 585 590
 Arg Asp Gly Arg Tyr Thr Pro Cys Ser Tyr Arg Gly Met Glu Glu Arg
 595 600 605
 Leu Pro His Gly Ser Met Ser Arg Leu Thr Asp His Ser Arg His Ser

610	615	620
Ser Ser His Arg Leu Asn Glu Gln Ser Arg His Ser Ser Ile Arg Asp		
625	630	635
Leu Ser Asn Asn Pro Met Thr His Ile Thr His Gly Thr Ser Met Asn		
	645	650
		655
Arg Val Ile Glu Glu Asp Gly Thr Ser Ala Glx		
	660	665

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1770 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: *Caenorhabditis elegans* putative transmembrane receptor (frizzled 1) gene, Coding region: 57..1634

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTCGGTT TAATTACCCA AGTTTGAGCT GTGAGCCCCC AATTCCATTA TCATTAATGG	60
GACCATTTTCG TGGTTACCTC GGAGTAACCT GGCTCCTGTT GCTCTTTGTG ATTGGTGTGG	120
ACGGGCAGAG GTGTCAAAAG GTGGATCATG AGATGTGCAA CGATTTGCCG TATAACTTAA	180
CGAGCTTCCC AAATCTCGTC GACGAGGAAT CATGGAAAGA CGCCTCCGAA TCCATCCTCA	240
CCTACAAGCC CCTGCTCTCC GTTGTCTGCT CCGAGCAGCT CAAATTCTTC CTGTGCTCCG	300
TCTACTTCCC GATGTGCAAC GAGAACTAG CCAACCCAAT TGGTCCATGC CGTCCATTGT	360
GTCTTTCCGT CCAGGAAAAG TGTCTTCCAG TGCTGGAAAG TTTCGGTTTC AAGTGGCCCCG	420
ATGTGATTCG TTGTGATAAG TTCCCGTTGG AGAACAATCG AGAGAAAATG TGCATGAAAG	480
GGCCAAATGA GCAAGGAGCA ATTCAAGATG AGAGGGCAAA GTTTGCAGCG AAAGAAAGTG	540
AGGACGACGG TAATGATCGA GTAGAAGATA TTCAACGGGA GGTCGACCGC CTCAACGGAA	600
AATGCCCACA GGATGAGGTG TTCCTGAATC GATCCTCAA GTGTGTGCCT TTGTGCTCGA	660
ACCCACAGAA GGTGGGCAG ACTGACCGTG AATCCGCCAC CCGACTCTTG TTGTTTCTCT	720
CGCTGAGCTC TGTAATACTA ACAATTCTAT CAGTCTTCAT AGTCGGCTTA TCACGTCTCG	780
AGATGCTCCA CTCACTTACG GAAACTGCCA TGTTCTTCTC GTGCATCTCG TTTTGTGCGA	840
CATCGGTTAT TTATATTGTG AGCATTTCGT TTAAAGATCA GTTCCAAATC TCGTGCACCG	900
ACTACACCCA TCACCTGCTC TTCGTCGTCG GAGGGCTTTC CCATGTTCCA TGTTCTTCAG	960
TGGCCTCACT GATTACTAC ACGGCAACTT GCTCACGTCT CTGGTGGCTC TTGATCTGTG	1020

```

TGTCGTGGAA TAAGGCGACA AGGACATCGC ATATATTGGA CGACTCCAGA ACCCGCGTGA      1080
TCATGCTCAT CCTGGGAATC CCGCTGGCTC CACTAATGCT CGCGCTACTC GCAAAAGCCG      1140
TCGCCGCCAA TCCCCTCACC GGACTCTGCT TCATCGGAGC AGCAAGCCCG GGCACCGACT      1200
GGATCTTCAA CTTCTGCCGG GAGCTCATTC TATTCCTCAT CAGCTCCATT GCTCTTTCGT      1260
CTGCTTGCTG CCGGCTTCTG GGCTCTGATG AGCAGGATGT CAATGGGTTT GCCGGAGTCA      1320
TTGCGGCAGT CTATCCGATT GCTGGACTAT TCTACATGCT TTCATTTGTG AACGATGCCA      1380
CCCAACCGTT TCTCTCACTT GACAGAAGTT TCAATGCGGT CTCGGCGACC AAGTTCTCGT      1440
TTGATCTACT TTTGAGCTTC ATCATGTGCG CGTTTTGTCT TATTTACTTG CTGTTCAAGC      1500
TGACTAGATC CTCATCAAAA GTTAGCAAAG AAGGATATCA ACCGGCGGTG CCGAAACTCC      1560
CGCAACCGGC AATTCCCGGC AGTGACGTT CGAACACCTA CGCGTCGACG TTTCGAACTA      1620
ATAATATGAT TTGAAGGATT TTCAATAATT TTTTGTGAAA AACAACGGGT TTATATAGAT      1680
AGAAAACAAA AAGGTGGTCT CAATTTTTTT TCCGTGAAAA TAAATTTTTA TTGATTTTTA      1740
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA      1770

```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 526 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Cfz1 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Gly Pro Phe Arg Gly Tyr Leu Gly Val Thr Trp Leu Leu Leu Leu
 1              5              10              15
Phe Val Ile Gly Val Asp Gly Gln Arg Cys Gln Lys Val Asp His Glu
      20              25              30
Met Cys Asn Asp Leu Pro Tyr Asn Leu Thr Ser Phe Pro Asn Leu Val
      35              40              45
Asp Glu Glu Ser Trp Lys Asp Ala Ser Glu Ser Ile Leu Thr Tyr Lys
      50              55              60
Pro Leu Leu Ser Val Val Cys Ser Glu Gln Leu Lys Phe Phe Leu Cys
      65              70              75              80
Ser Val Tyr Phe Pro Met Cys Asn Glu Lys Leu Ala Asn Pro Ile Gly
      85              90              95
Pro Cys Arg Pro Leu Cys Leu Ser Val Gln Glu Lys Cys Leu Pro Val
      100              105              110

```


Leu Glu Ser Phe Gly Phe Lys Trp Pro Asp Val Ile Arg Cys Asp Lys
 115 120 125
 Phe Pro Leu Glu Asn Asn Arg Glu Lys Met Cys Met Lys Gly Pro Asn
 130 135 140
 Glu Gln Gly Ala Ile Gln Asp Glu Arg Ala Lys Phe Ala Ala Lys Glu
 145 150 155 160
 Ser Glu Asp Asp Gly Asn Asp Arg Val Glu Asp Ile Gln Arg Glu Val
 165 170 175
 Asp Arg Leu Asn Gly Lys Cys Pro Gln Asp Glu Val Phe Leu Asn Arg
 180 185 190
 Ser Ser Lys Cys Val Pro Leu Cys Ser Asn Pro Gln Lys Val Gly Gln
 195 200 205
 Thr Asp Arg Glu Ser Ala Thr Arg Leu Leu Leu Phe Leu Ser Leu Ser
 210 215 220
 Ser Val Ile Leu Thr Ile Leu Ser Val Phe Ile Val Gly Leu Ser Arg
 225 230 235 240
 Leu Glu Met Leu His Ser Leu Thr Glu Thr Ala Met Phe Phe Ser Cys
 245 250 255
 Ile Ser Phe Cys Ala Thr Ser Val Ile Tyr Ile Val Ser Ile Ser Phe
 260 265 270
 Lys Asp Gln Phe Gln Ile Ser Cys Thr Asp Tyr Thr His His Leu Leu
 275 280 285
 Phe Val Val Gly Gly Leu Ser His Val Pro Cys Ser Ser Val Ala Ser
 290 295 300
 Leu Ile Tyr Tyr Thr Ala Thr Cys Ser Arg Leu Trp Trp Leu Leu Ile
 305 310 315 320
 Cys Val Ser Trp Asn Lys Ala Thr Arg Thr Ser His Ile Leu Asp Asp
 325 330 335
 Ser Arg Thr Arg Val Ile Met Leu Ile Leu Gly Ile Pro Leu Ala Pro
 340 345 350
 Leu Met Leu Ala Leu Leu Ala Lys Ala Val Ala Ala Asn Pro Leu Thr
 355 360 365
 Gly Leu Cys Phe Ile Gly Ala Ala Ser Pro Gly Thr Asp Trp Ile Phe
 370 375 380
 Asn Phe Cys Arg Glu Leu Ile Leu Phe Leu Ile Ser Ser Ile Ala Leu
 385 390 395 400
 Ser Ser Ala Cys Cys Arg Leu Leu Gly Ser Asp Glu Gln Asp Val Asn
 405 410 415
 Gly Phe Ala Gly Val Ile Ala Ala Val Tyr Pro Ile Ala Gly Leu Phe
 420 425 430
 Tyr Met Leu Ser Phe Val Asn Asp Ala Thr Gln Pro Phe Leu Ser Leu
 435 440 445
 Asp Arg Ser Phe Asn Ala Val Ser Ala Thr Lys Phe Ser Phe Asp Leu
 450 455 460
 Leu Leu Ser Phe Ile Met Cys Ala Phe Cys Leu Ile Tyr Leu Leu Phe

465		470		475		480									
Lys	Leu	Thr	Arg	Ser	Ser	Ser	Lys	Val	Ser	Lys	Glu	Gly	Tyr	Gln	Pro
				485					490					495	
Ala	Val	Pro	Lys	Leu	Pro	Gln	Pro	Ala	Ile	Pro	Gly	Ser	Val	Arg	Ser
			500					505					510		
Asn	Thr	Tyr	Ala	Ser	Thr	Phe	Arg	Thr	Asn	Asn	Met	Ile	Glx		
		515					520					525			

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2828 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: Mus musculus putative transmembrane receptor (frizzled 4) mRNA,
Coding region: 238..1941

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCGACCTCAA	CACAAAGACC	TGGGTCGTGA	GACACACGCG	TAGAGTCAGG	CGGCTTCCCC	60
GAAAACCGGA	CTCGGCCGGC	GCCGAGTCTG	GGTCCCCGCC	TTCAACCATG	ACCCTAGCAA	120
TCCATCCCTC	GGCCCCGGGCT	CCGGACGTCT	GATATTCCGC	ACATTCTCGT	ACAAGTGTCTG	180
GAGAGGCGAC	TGCTGCCCCC	TTGTCGCCCT	TGGCGCCTTA	CCGCATTCCC	TATCCGGAGT	240
TGGGAGCAGC	GCGGCCACCG	GCGCCCCTGT	GCAAAGTGGG	GGTGTCTGCT	AGATCAGCCT	300
CTGCCGCTGC	TGCCCCGAGC	TCTGGCCATG	GCCTGGCCGG	GCACAGGGCC	GAGCAGCCGG	360
GGGGCGCCTG	GAGGCGTCGG	GCTCAGGCTG	GGGCTGCTGC	TGCAGTTCCT	CCTGCTCCTG	420
CGGCCGACAC	TGGGGTTTCG	GGACGAGGAG	GAGCGGCGCT	GCGACCCCAT	CCGCATCGCC	480
ATGTGCCAGA	ACCTCGGCTA	CAACGTGACC	AAGATGCCCA	ACTTAGTGGG	ACACGAGCTG	540
CAGACAGACG	CCGAGCTGCA	GCTGACAACT	TTCACGCCGC	TCATCCAGTA	CGGCTGCTCC	600
AGCCAGCTGC	AGTTCTTCCT	TTGTTTCGGT	TATGTGCCAA	TGTGCACAGA	GAAGATCAAC	660
ATCCCCATCG	GCCCCGTGCG	TGGCATGTGC	CTTTCAGTCA	AGAGACGCTG	TGAACAGTC	720
CTGAGAGAAT	TTGGGTTTGC	CTGGCCCGAC	ACCCTGAACT	GCAGCAAGTT	CCCGCCCCAG	780
AACGACCACA	ACCACATGTG	CATGGAAGGA	CCAGGTGATG	AAGAGGTTCC	CTTGCCCCAC	840
AAGACTCCCA	TCCAGCCCGG	GGAAGAGTGC	CACTCCGTGG	GAAGCAATTC	TGATCAGTAC	900
ATCTGGGTGA	AGAGGAGCCT	GAAGTGTGTT	CTCAAGTGTG	GCTACGATGC	TGGCTTGTAC	960
AGCCGCTCAG	CTAAGGAGTT	CACGGATATT	TGGATGGCTG	TGTGGGCCAG	CCTCTGCTTC	1020

ATCTCCACCA	CCTTCACCGT	GCTGACCTTC	CTGATTGATT	CATCCAGGTT	TTCTTACCCT	1080
GAGCGCCCCA	TCATATTTCT	CAGTATGTGC	TATAATATTT	ATAGCATTGC	TTATATTGTT	1140
CGGCTGACTG	TAGGCCGGGA	AAGGATATCC	TGTGATTTTG	AAGAGGCGGC	AGAGCCCGTT	1200
CTCATCCAAG	AAGGACTTAA	GAACACAGGA	TGTGCAATAA	TTTTCTTGCT	GATGTACTTT	1260
TTTGGAATGG	CCAGCTCCAT	TTGGTGGGTT	ATTCTGACAC	TCACTTGGTT	TTTGGCAGCC	1320
GGACTCAAGT	GGGGTCATGA	AGCCATTGAA	ATGCACAGTT	CTTATTTCCA	CATCGCAGCC	1380
TGGGCTATTC	CCGCAGTGAA	AACCATTGTC	ATCTTGATTA	TGAGACTAGT	GGATGCCGAT	1440
GAAGTGAAGT	GCTTGTGCTA	TGTTGGGAAC	CAAAACCTAG	ATGCCCTCAC	TGGCTTTGTG	1500
GTGGCTCCTC	TCTTTACGTA	TTTGGTGATT	GGAACGCTGT	TCATTGCGGC	GGGTTTGGTG	1560
GCCTTATTCA	AAATTCGGTC	CAATCTTCAA	AAAGACGGGA	CAAAGACAGA	CAAGTTGGAA	1620
AGGCTAATGG	TCAAGATCGG	GGTCTTCTCA	GTACTGTACA	CGGTTCCCTGC	AACCTGTGTG	1680
ATTGCCTGTT	ATTTCTATGA	AATCTCAAAC	TGGGCACTCT	TTGATATTC	TGCAGATGAC	1740
TCAAACATGG	CAGTTGAAAT	GTTGAAAATT	TTTATGTCTT	TGCTCGTGGG	CATCACTTCA	1800
GGCATGTGGA	TTTGGTCTGC	CAAAACTCTT	CACACGTGGC	AAAAGTGTTT	TAACCGATTG	1860
GTGAATTCTG	GGAAGGTAAA	GAGAGAGAAG	AGGGGGAATG	GTTGGGTGAA	GCCAGGAAAA	1920
GGCAACGAGA	CTGTGGTATA	AGACTAGCCG	GCTTCCTCGT	TCCTCATTGT	GAAGGAAGTG	1980
ATGCAGGGAA	TCTCAGTTTG	AACAAACTTA	GAAACACTTC	AGCCCACACA	CACCCACGTC	2040
AGCCCACCAC	CACTCACCCA	ACTCAGCATC	AGAAGACCAA	TGGCTTCACT	GCAGACTTTG	2100
GAATGGTCCA	AAATGGAAAA	GCCAGTTAAG	AGGTTTTTCAA	AGCTGTGAAA	AATCAAAATG	2160
TTGATCACTT	TAGCAGGTCA	CAGCTTGAG	TCCGTGGAGG	TCCCGCCTAG	ATTCTCTGAAG	2220
CCCAGGGTGA	TAGTGTGTTG	TCCTACTGGG	TGGGATTTCA	ACTGTGAGTT	GATAACATGC	2280
AAGGAGAAAG	ATTAATTTTT	AAAACCCCTT	TAAATTTTAA	ATAGTAACTA	AGGTCTTGCA	2340
GATAGCAAAG	TGATCTATAA	ACACTGGAAA	TGCTGGGTTG	GGAGACGTGT	TGCAGAGTTT	2400
TTATATGTTT	CTGGTCTAAC	ATAAACATCT	TCTGGCCTAC	ACTGTCTGCT	GTTTAGAACT	2460
CTGTAGCGCA	CTCCCAGAGG	TGGTGTCAAA	ATCCTTCAGT	GCCTTGTCGT	AAAACAGAAT	2520
TGTTTGAGCA	AACAAAAGTA	CTGTACTAAC	ACACGTAAGG	TATCCAGTGG	ATTTCTCTCT	2580
CCTGAAATTT	CAACATCCCT	AATTCTAGGC	AGCCCCTGTT	TTCTTCACTT	TAAACTAATG	2640
ACTCAAAAAA	AAAAAGGTTA	TTTTTATAGG	ATTTTTTTTT	GCACTGCAGC	ATGCCTAATG	2700
AGAGGAAAAG	GAGGTGATCA	CTTCTGACAA	TCACTTAATT	CAGAGAAAAA	TGAGATTTGC	2760
TAATTGACTT	ACCTTCCGAC	CCCTAGAGAC	CCTATTGCAT	TAAGCAATGT	TTAAGCAATT	2820
GGGGACTT						2828

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 538 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Mfz4 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Ala	Trp	Pro	Gly	Thr	Gly	Pro	Ser	Ser	Arg	Gly	Ala	Pro	Gly	Gly	1	5	10	15
Val	Gly	Leu	Arg	Leu	Gly	Leu	Leu	Leu	Gln	Phe	Leu	Leu	Leu	Leu	Arg	20	25	30	
Pro	Thr	Leu	Gly	Phe	Gly	Asp	Glu	Glu	Glu	Arg	Arg	Cys	Asp	Pro	Ile	35	40	45	
Arg	Ile	Ala	Met	Cys	Gln	Asn	Leu	Gly	Tyr	Asn	Val	Thr	Lys	Met	Pro	50	55	60	
Asn	Leu	Val	Gly	His	Glu	Leu	Gln	Thr	Asp	Ala	Glu	Leu	Gln	Leu	Thr	65	70	75	80
Thr	Phe	Thr	Pro	Leu	Ile	Gln	Tyr	Gly	Cys	Ser	Ser	Gln	Leu	Gln	Phe	85	90	95	
Phe	Leu	Cys	Ser	Val	Tyr	Val	Pro	Met	Cys	Thr	Glu	Lys	Ile	Asn	Ile	100	105	110	
Pro	Ile	Gly	Pro	Cys	Gly	Gly	Met	Cys	Leu	Ser	Val	Lys	Arg	Arg	Cys	115	120	125	
Glu	Pro	Val	Leu	Arg	Glu	Phe	Gly	Phe	Ala	Trp	Pro	Asp	Thr	Leu	Asn	130	135	140	
Cys	Ser	Lys	Phe	Pro	Pro	Gln	Asn	Asp	His	Asn	His	Met	Cys	Met	Glu	145	150	155	160
Gly	Pro	Gly	Asp	Glu	Glu	Val	Pro	Leu	Pro	His	Lys	Thr	Pro	Ile	Gln	165	170	175	
Pro	Gly	Glu	Glu	Cys	His	Ser	Val	Gly	Ser	Asn	Ser	Asp	Gln	Tyr	Ile	180	185	190	
Trp	Val	Lys	Arg	Ser	Leu	Asn	Cys	Val	Leu	Lys	Cys	Gly	Tyr	Asp	Ala	195	200	205	
Gly	Leu	Tyr	Ser	Arg	Ser	Ala	Lys	Glu	Phe	Thr	Asp	Ile	Trp	Met	Ala	210	215	220	
Val	Trp	Ala	Ser	Leu	Cys	Phe	Ile	Ser	Thr	Thr	Phe	Thr	Val	Leu	Thr	225	230	235	240
Phe	Leu	Ile	Asp	Ser	Ser	Arg	Phe	Ser	Tyr	Pro	Glu	Arg	Pro	Ile	Ile	245	250	255	
Phe	Leu	Ser	Met	Cys	Tyr	Asn	Ile	Tyr	Ser	Ile	Ala	Tyr	Ile	Val	Arg	260	265	270	
Leu	Thr	Val	Gly	Arg	Glu	Arg	Ile	Ser	Cys	Asp	Phe	Glu	Glu	Ala	Ala				

35

275					280					285					
Glu	Pro	Val	Leu	Ile	Gln	Glu	Gly	Leu	Lys	Asn	Thr	Gly	Cys	Ala	Ile
290					295					300					
Ile	Phe	Leu	Leu	Met	Tyr	Phe	Phe	Gly	Met	Ala	Ser	Ser	Ile	Trp	Trp
305					310					315					320
Val	Ile	Leu	Thr	Leu	Thr	Trp	Phe	Leu	Ala	Ala	Gly	Leu	Lys	Trp	Gly
				325					330					335	
His	Glu	Ala	Ile	Glu	Met	His	Ser	Ser	Tyr	Phe	His	Ile	Ala	Ala	Trp
			340					345					350		
Ala	Ile	Pro	Ala	Val	Lys	Thr	Ile	Val	Ile	Leu	Ile	Met	Arg	Leu	Val
		355					360					365			
Asp	Ala	Asp	Glu	Leu	Thr	Gly	Leu	Cys	Tyr	Val	Gly	Asn	Gln	Asn	Leu
	370					375					380				
Asp	Ala	Leu	Thr	Gly	Phe	Val	Val	Ala	Pro	Leu	Phe	Thr	Tyr	Leu	Val
385					390					395					400
Ile	Gly	Thr	Leu	Phe	Ile	Ala	Ala	Gly	Leu	Val	Ala	Leu	Phe	Lys	Ile
				405					410					415	
Arg	Ser	Asn	Leu	Gln	Lys	Asp	Gly	Thr	Lys	Thr	Asp	Lys	Leu	Glu	Arg
			420					425					430		
Leu	Met	Val	Lys	Ile	Gly	Val	Phe	Ser	Val	Leu	Tyr	Thr	Val	Pro	Ala
		435					440					445			
Thr	Cys	Val	Ile	Ala	Cys	Tyr	Phe	Tyr	Glu	Ile	Ser	Asn	Trp	Ala	Leu
	450					455					460				
Phe	Arg	Tyr	Ser	Ala	Asp	Asp	Ser	Asn	Met	Ala	Val	Glu	Met	Leu	Lys
465					470					475					480
Ile	Phe	Met	Ser	Leu	Leu	Val	Gly	Ile	Thr	Ser	Gly	Met	Trp	Ile	Trp
				485					490					495	
Ser	Ala	Lys	Thr	Leu	His	Thr	Trp	Gln	Lys	Cys	Ser	Asn	Arg	Leu	Val
			500					505					510		
Asn	Ser	Gly	Lys	Val	Lys	Arg	Glu	Lys	Arg	Gly	Asn	Gly	Trp	Val	Lys
		515					520					525			
Pro	Gly	Lys	Gly	Asn	Glu	Thr	Val	Val	Glx						
	530					535									

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2334 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: mRNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: Human transmembrane receptor
(frizzled 5) mRNA, Coding region: 321..2078

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACCCAGGGAC	GGAGGACCCA	GGCTGGCTTG	GGGACTGTCT	GCTCTTCTCG	GCGGGAGCCG	60
TGGAGAGTCC	TTTCCCTGGA	ATCCGAGCCC	TAACCGTCTC	TCCCCAGCCC	TATCCGGCGA	120
GGAGCGGAGC	GCTGCCAGCG	GAGGCAGCGC	CTTCCCCAAG	CAGTTTATCT	TTGGACGGTT	180
TTCTTTAAAG	GAAAAACGAA	CCAACAGGTT	GCCAGCCCCG	GCGCCACACA	CGAGACGCCG	240
GAGGGAGAAG	CCCCGGCCCC	GATTCCTCTG	CCTGTGTGCG	TCCCTCGCGG	GCTGCTGGAG	300
GCGAGGGGAG	GGAGGGGGCG	ATGGCTCGGC	CTGACCCATC	CGCGCCGCCC	TCGCTGTTGC	360
TGCTGCTCCT	GGCGCAGCTG	GTGGGCCCGG	CGGCCGCCGC	GTCCAAGGCC	CCGGTGTGCC	420
AGGAAATCAC	GGTGCCCATG	TGCCGCGGCA	TCGGCTACAA	CCTGACGCAC	ATGCCCAACC	480
AGTTCAACCA	CGACACGCAG	GACGAGGCGG	GCCTGGAGGT	GCACCAGTTC	TGGCCGCTGG	540
TGGAGATCCA	ATGCTCGCCG	GACCTGCGCT	TCTTCCTATG	CACTATGTAC	ACGCCCATCT	600
GTCTGCCCCA	CTACCACAAG	CCGCTGCCGC	CCTGCCGCTC	GGTGTGCGAG	CGCGCCAAGG	660
CCGGCTGCTC	GCCGCTGATG	CGCCAGTACG	GCTTCGCCTG	GCCCCAGCGC	ATGAGCTGCG	720
ACCGCCTCCC	GGTGCTGGGC	CGCGACGCCG	AGGTCCCTCTG	CATGGATTAC	AACCGCAGCG	780
AGGCCACCAC	GGCGCCCCCC	AGGCCTTTCC	CAGCCAAGCC	CACCCTTCCA	GGCCCCCCAG	840
GGGCGCCGGC	CTCGGGGGGC	GAATGCCCCG	CTGGGGGGCC	GTTCTGTGTC	AAGTGTGCGG	900
AGCCCTTCGT	GCCCATTTCTG	AAGGAGTCAC	ACCCGCTCTA	CAACAAGGTG	CGGACGGGCC	960
AGGTGCCCAA	CTGCGCGGTA	CCCTGCTACC	AGCCGTCCTT	CAGTGCCGAC	GAGCGCACGT	1020
TCGCCACCTT	CTGGATAGGC	CTGTGGTCGG	TGCTGTGCTT	CATCTCCACG	TCCACCACAG	1080
TGGCCACCTT	CCTCATCGAC	ATGGACACGT	TCCGCTATCC	TGAGCGCCCC	ATCATCTTCC	1140
TGTCAGCCTG	CTACCTGTGC	GTGTCGCTGG	GCTTCCTGGT	GCGTCTGGTC	GTGGGGCCATG	1200
CCAGCGTGGC	CTGCAGCCGC	GAGCACAACC	ACATCCACTA	CGAGACCACG	GGCCCTGCAC	1260
TGTGCACCAT	CGTCTTCCTC	CTGGTCTACT	TCTTCGGCAT	GGCCAGCTCC	ATCTGGTGGG	1320
TCATCCTGTC	GCTCACCTGG	TTCTTGCCCG	CCGCGATGAA	GTGGGGCAAC	GAGGCCATCG	1380
CGGGCTACGG	CCAGTACTTC	CACCTGGCTG	CGTGGCTCAT	CCCCAGCGTC	AAGTCCATCA	1440
CGGCACTGGC	GCTGAGCTCC	GTGGACGGGG	ACCCAGTGGC	CGGCATCTGC	TACGTGGGCA	1500
ACCAGAACCT	GAACTCGCTG	CGGCGCTTCG	TGCTGGGCCC	GCTGGTGCTC	TACCTGCTGG	1560
TGGGCACGCT	CTTCCTGCTG	GCGGGCTTCG	TGTCGCTCTT	CCGCATCCGC	AGCGTCATCA	1620
AGCAGGGCGG	CACCAAGACG	GACAAGCTGG	AGAAGCTCAT	GATCCGCATC	GGCATCTTCA	1680
CGCTGCTCTA	CACGGTCCCC	GCCAGCATTG	TGGTGGCCTG	CTACCTGTAC	GAGCAGCACT	1740
ACCGCGAGAG	CTGGGAGGCG	GCGCTCACCT	GCGCCTGCCC	GGGCCACGAC	ACCGGCCAGC	1800
CGCGCGCCAA	GCCCCAGTAC	TGGGTGCTCA	TGCTCAAGTA	CTTCATGTGC	CTGGTGGTGG	1860
GCATCACGTC	GGGCGTCTGG	ATCTGGTCGG	GCAAGACGGT	GGAGTCGTGG	CGGCGTTTCA	1920

```

CCAGCCGCTG CTGCTGCCGC CCGCGGCGCG GCCACAAGAG CGGGGGCGCC ATGGCCGCAG      1980
GGGACTACCC CGAGGCGAGC GCCGCGCTCA CAGGCAGGAC CGGGCCGCCG GGCCCCGCCG      2040
CCACCTACCA CAAGCAGGTG TCCCTGTCGC ACGTGTAGGA GGCTGCCGCC GAGGGACTCG      2100
GCCGGAGAGC TGAGGGGAGG GGGGCGTTTT GTTTGGTAGT TTTGCCAAGG TCACTTCCGT      2160
TTACCTTCAT GGTGCTGTTG CCCCTCCCCG CGGCGACTTG GAGAGAGGGA AGAGGGGCGT      2220
TTTCGAGGAA GAACCTGTCC CAGGTCTTCT CCAAGGGGCC CAGCTCACGT GTATTCTATT      2280
TTGCGTTTCT TACCTGCCTT CTTTATGGGA ACCCTCTTTT TAATTTATAT GTAT          2334

```

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 586 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: Hfz5 protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met	Ala	Arg	Pro	Asp	Pro	Ser	Ala	Pro	Pro	Ser	Leu	Leu	Leu	Leu	Leu	1	5	10	15
Leu	Ala	Gln	Leu	Val	Gly	Arg	Ala	Ala	Ala	Ser	Lys	Ala	Pro	Val		20	25	30	
Cys	Gln	Glu	Ile	Thr	Val	Pro	Met	Cys	Arg	Gly	Ile	Gly	Tyr	Asn	Leu	35	40	45	
Thr	His	Met	Pro	Asn	Gln	Phe	Asn	His	Asp	Thr	Gln	Asp	Glu	Ala	Gly	50	55	60	
Leu	Glu	Val	His	Gln	Phe	Trp	Pro	Leu	Val	Glu	Ile	Gln	Cys	Ser	Pro	65	70	75	80
Asp	Leu	Arg	Phe	Phe	Leu	Cys	Thr	Met	Tyr	Thr	Pro	Ile	Cys	Leu	Pro	85	90	95	
Asp	Tyr	His	Lys	Pro	Leu	Pro	Pro	Cys	Arg	Ser	Val	Cys	Glu	Arg	Ala	100	105	110	
Lys	Ala	Gly	Cys	Ser	Pro	Leu	Met	Arg	Gln	Tyr	Gly	Phe	Ala	Trp	Pro	115	120	125	
Glu	Arg	Met	Ser	Cys	Asp	Arg	Leu	Pro	Val	Leu	Gly	Arg	Asp	Ala	Glu	130	135	140	
Val	Leu	Cys	Met	Asp	Tyr	Asn	Arg	Ser	Glu	Ala	Thr	Thr	Ala	Pro	Pro	145	150	155	160
Arg	Pro	Phe	Pro	Ala	Lys	Pro	Thr	Leu	Pro	Gly	Pro	Pro	Gly	Ala	Pro	165	170	175	

38

Ala	Ser	Gly	Gly	Glu	Cys	Pro	Ala	Gly	Gly	Pro	Phe	Val	Cys	Lys	Cys		
			180					185					190				
Arg	Glu	Pro	Phe	Val	Pro	Ile	Leu	Lys	Glu	Ser	His	Pro	Leu	Tyr	Asn		
		195					200					205					
Lys	Val	Arg	Thr	Gly	Gln	Val	Pro	Asn	Cys	Ala	Val	Pro	Cys	Tyr	Gln		
	210					215					220						
Pro	Ser	Phe	Ser	Ala	Asp	Glu	Arg	Thr	Phe	Ala	Thr	Phe	Trp	Ile	Gly		
225					230					235					240		
Leu	Trp	Ser	Val	Leu	Cys	Phe	Ile	Ser	Thr	Ser	Thr	Thr	Val	Ala	Thr		
				245					250					255			
Phe	Leu	Ile	Asp	Met	Asp	Thr	Phe	Arg	Tyr	Pro	Glu	Arg	Pro	Ile	Ile		
			260					265					270				
Phe	Leu	Ser	Ala	Cys	Tyr	Leu	Cys	Val	Ser	Leu	Gly	Phe	Leu	Val	Arg		
		275					280					285					
Leu	Val	Val	Gly	His	Ala	Ser	Val	Ala	Cys	Ser	Arg	Glu	His	Asn	His		
	290					295					300						
Ile	His	Tyr	Glu	Thr	Thr	Gly	Pro	Ala	Leu	Cys	Thr	Ile	Val	Phe	Leu		
305					310					315					320		
Leu	Val	Tyr	Phe	Phe	Gly	Met	Ala	Ser	Ser	Ile	Trp	Trp	Val	Ile	Leu		
				325					330					335			
Ser	Leu	Thr	Trp	Phe	Leu	Ala	Ala	Ala	Met	Lys	Trp	Gly	Asn	Glu	Ala		
			340				345						350				
Ile	Ala	Gly	Tyr	Gly	Gln	Tyr	Phe	His	Leu	Ala	Ala	Trp	Leu	Ile	Pro		
		355					360					365					
Ser	Val	Lys	Ser	Ile	Thr	Ala	Leu	Ala	Leu	Ser	Ser	Val	Asp	Gly	Asp		
	370					375					380						
Pro	Val	Ala	Gly	Ile	Cys	Tyr	Val	Gly	Asn	Gln	Asn	Leu	Asn	Ser	Leu		
385					390				395						400		
Arg	Arg	Phe	Val	Leu	Gly	Pro	Leu	Val	Leu	Tyr	Leu	Leu	Val	Gly	Thr		
				405					410					415			
Leu	Phe	Leu	Leu	Ala	Gly	Phe	Val	Ser	Leu	Phe	Arg	Ile	Arg	Ser	Val		
			420					425					430				
Ile	Lys	Gln	Gly	Gly	Thr	Lys	Thr	Asp	Lys	Leu	Glu	Lys	Leu	Met	Ile		
		435					440					445					
Arg	Ile	Gly	Ile	Phe	Thr	Leu	Leu	Tyr	Thr	Val	Pro	Ala	Ser	Ile	Val		
	450					455					460						
Val	Ala	Cys	Tyr	Leu	Tyr	Glu	Gln	His	Tyr	Arg	Glu	Ser	Trp	Glu	Ala		
465					470					475					480		
Ala	Leu	Thr	Cys	Ala	Cys	Pro	Gly	His	Asp	Thr	Gly	Gln	Pro	Arg	Ala		
				485					490					495			
Lys	Pro	Glu	Tyr	Trp	Val	Leu	Met	Leu	Lys	Tyr	Phe	Met	Cys	Leu	Val		
			500					505					510				
Val	Gly	Ile	Thr	Ser	Gly	Val	Trp	Ile	Trp	Ser	Gly	Lys	Thr	Val	Glu		
		515					520					525					
Ser	Trp	Arg	Arg	Phe	Thr	Ser	Arg	Cys	Cys	Cys	Arg	Pro	Arg	Arg	Gly		

530	535	540
His Lys Ser Gly Gly Ala Met Ala Ala Gly Asp Tyr Pro Glu Ala Ser		
545	550	555 560
Ala Ala Leu Thr Gly Arg Thr Gly Pro Pro Gly Pro Ala Ala Thr Tyr		
	565	570 575
His Lys Gln Val Ser Leu Ser His Val Glx		
	580	585

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2492 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: Mus musculus putative transmembrane receptor (frizzled 6) mRNA, Coding region: 146..2275

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCATTTTCAGG CCCAGCTACT ATCAAAATGG TACAAAGAAT GCAATGAGGA ATTTGTACAT	60
TTTATCTCTG ATTTGAGAAT CTTTTTGATG CGGAAAGGAG CATAAGAATA ATCCAAGCCA	120
TGTGGTAAAA TCGGAGTCTG GCAAGATGGA AAGGTCCCCG TTTCTGTTGG CGTGCATTCT	180
TCTGCCCCCTC GTAAGAGGAC ACAGCCTTTT CACCTGTGAG CCAATCACCG TTCCCAGATG	240
TATGAAAATG ACTTACAACA TGACGTTCTT CCCTAACCTG ATGGGTCATT ATGACCAGGG	300
GATCGCTGCT GTGGAAATGG GGCACCTTCT GCATCTTGCA AATCTAGAAT GTTCACCAAA	360
CATTGAAATG TTCCTTTGCC AAGCTTTTAT ACCAACCTGC ACAGAGCAAA TTCATGTAGT	420
TCTACCCTGT CGGAAATTGT GTGAGAAAAT AGTTTCTGAT TGCAAAAAAC TAATGGACAC	480
TTTTGGCATC CGATGGCCTG AAGAACTTGA ATGTAACAGA TTGCCCACT GTGATGACAC	540
TGTTCTTGTA ACTTCTCATC CACACACAGA GCTTTCTGGG CCACAGAAGA AATCAGATCA	600
AGTCCCAAGA GACATTGGAT TTTGGTGTCC AAAGCACCTT AGGACTTCCG GGGACCAAGG	660
CTATAGGTTT CTGGGAATTG AACAGTGTGC CCCTCCGTGC CCCAATATGT ATTTTAAAAG	720
TGATGAACTA GACTTTGCCA AAAGTTTCAT AGGAATAGTT TCAATATTTT GTCTTTGTGC	780
AACTCTGTTC ACGTTCCTTA CATTTTTAAT TGACGTTAGA CGATTCAGAT ACCCAGAGAG	840
ACCAATTATC TATTACTCTG TCTGCTACAG CATTGTCTCT CTCATGTACT TCGTGGGGTT	900
TTTGCTGGGC AATAGCACAG CTTGTAATAA GGCAGACGAG AAGCTGGAGC TCGGGGACAC	960
CGTTGTCCTA GGGTCAAAGA ATAAGGCTTG CAGTGTGGTA TTTATGTTTC TGTATTTTTT	1020
TACAATGGCT GGCACCGTGT GGTGGGTGAT TCTCACCATT ACGTGGTTCT TAGCTGCCCG	1080

GAGAAAATGG	AGTTGCGAAG	CTATTGAACA	AAAAGCAGTG	TGGTTCCATG	CCGTTGCCTG	1140
GGGGGCGCCC	GGGTTCTGA	CCGTCATGCT	GCTCGCTATG	AATAAGGTTG	AAGGAGACAA	1200
CATTAGCGGC	GTTTGCTTCG	TTGGCCTGTA	TGACCTGGAC	GCCTCTCGCT	ACTTCGTCTT	1260
TCTGCCTCTG	TGCCTCTGCG	TATTTGTTGG	GCTGTCTCTC	CTCTTAGCCG	GCATCATCTC	1320
CTTGAATCAT	GTCCGACAAG	TCATACAGCA	TGATGGTCGG	AACCAAGAGA	AGCTAAAGAA	1380
ATTCATGATT	CGCATCGGAG	TCTTCAGTGG	CCTGTATCTT	GTGCCCTTAG	TGACACTTCT	1440
CGGTTGCTAT	GTCTATGAGC	TAGTGAACAG	GATCACCTGG	GAGATGACAT	GGTTCTCTGA	1500
TCATTGTCAC	CAGTACCGCA	TCCCGTGCCC	TTACCAGGCA	AATCCAAAAG	CTCGACCAGA	1560
ATTGGCTTTA	TTTATGATAA	AATATCTGAT	GACATTAATT	GTTGGTATCT	CTGCGGTCTT	1620
CTGGGTGGA	AGCAAAAAGA	CGTGACACAGA	ATGGGCCGGG	TTCTTTAAGC	GAAACCGCAA	1680
GCGAGACCCC	ATCAGTGAGA	GCCGCCGAGT	GCTGCAAGAG	TCCTGTGAGT	TCTTCCTGAA	1740
GCACAACTCT	AAAGTGAAGC	ACAAGAAGAA	GCATGGCGCA	CCAGGGCCTC	ATAGGCTGAA	1800
GGTCATTTC	AAGTCCATGG	GAAC TAGCAC	AGGAGCGACC	ACAAATCATG	GCACCTCTGC	1860
CATGGCAATC	GCTGACCATG	ATTACTTAGG	GCAAGAACT	TCAACAGAAG	TCCACACCTC	1920
CCCAGAAGCA	TCCGTCAAAG	AGGGACGAGC	AGACCGAGCA	AACACTCCCA	GCGCCAAAGA	1980
TCGGGACTGT	GGGGAATCTG	CAGGGCCCCAG	TTCCAAGCTC	TCTGGGAACC	GGAACGGCAG	2040
GGAAAGCCGA	GCGGGCGGCC	TGAAGGAGAG	AAGCAATGGA	TCAGAGGGGG	CTCCAAGTGA	2100
AGGAAGGGTA	AGTCCAAAGA	GCAGCGTTCC	TGAGACTGGC	CTGATAGACT	GCAGCACTTC	2160
ACAGGCCGCC	AGTTCTCCAG	AACCAACCAG	CCTCAAGGGC	TCCACATCTC	TGCCTGTTCA	2220
CTCAGCTTCC	AGAGCTAGGA	AAGAGCAGGG	TGCTGGCAGC	CATTCCGACG	CTTGAAGAAA	2280
ACTGTCTCGT	TCCCCCAGAA	GCACATGTAT	GTTACACTGG	AGATGACCAA	CTGATTTGTC	2340
TTATAAAGGC	CACTGTTGAG	CTGGGAGAGT	AGCCAGTGG	TACAGCGCCC	ACCTGGAATA	2400
CTGAGGACCT	GGGGTTGTCT	CCCAGCACTG	CAAAAGGAAA	ATTCACTGTT	ACAGTCTTCC	2460
TTGCACTTAA	CCAGCTTTGT	CTATGTTTTT	TT			2492

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 710 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: Mfz6 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Glu Arg Ser Pro Phe Leu Leu Ala Cys Ile Leu Leu Pro Leu Val
 1 5 10 15
 Arg Gly His Ser Leu Phe Thr Cys Glu Pro Ile Thr Val Pro Arg Cys
 20 25 30
 Met Lys Met Thr Tyr Asn Met Thr Phe Phe Pro Asn Leu Met Gly His
 35 40 45
 Tyr Asp Gln Gly Ile Ala Ala Val Glu Met Gly His Phe Leu His Leu
 50 55 60
 Ala Asn Leu Glu Cys Ser Pro Asn Ile Glu Met Phe Leu Cys Gln Ala
 65 70 75 80
 Phe Ile Pro Thr Cys Thr Glu Gln Ile His Val Val Leu Pro Cys Arg
 85 90 95
 Lys Leu Cys Glu Lys Ile Val Ser Asp Cys Lys Lys Leu Met Asp Thr
 100 105 110
 Phe Gly Ile Arg Trp Pro Glu Glu Leu Glu Cys Asn Arg Leu Pro His
 115 120 125
 Cys Asp Asp Thr Val Pro Val Thr Ser His Pro His Thr Glu Leu Ser
 130 135 140
 Gly Pro Gln Lys Lys Ser Asp Gln Val Pro Arg Asp Ile Gly Phe Trp
 145 150 155 160
 Cys Pro Lys His Leu Arg Thr Ser Gly Asp Gln Gly Tyr Arg Phe Leu
 165 170 175
 Gly Ile Glu Gln Cys Ala Pro Pro Cys Pro Asn Met Tyr Phe Lys Ser
 180 185 190
 Asp Glu Leu Asp Phe Ala Lys Ser Phe Ile Gly Ile Val Ser Ile Phe
 195 200 205
 Cys Leu Cys Ala Thr Leu Phe Thr Phe Leu Thr Phe Leu Ile Asp Val
 210 215 220
 Arg Arg Phe Arg Tyr Pro Glu Arg Pro Ile Ile Tyr Tyr Ser Val Cys
 225 230 235 240
 Tyr Ser Ile Val Ser Leu Met Tyr Phe Val Gly Phe Leu Leu Gly Asn
 245 250 255
 Ser Thr Ala Cys Asn Lys Ala Asp Glu Lys Leu Glu Leu Gly Asp Thr
 260 265 270
 Val Val Leu Gly Ser Lys Asn Lys Ala Cys Ser Val Val Phe Met Phe
 275 280 285
 Leu Tyr Phe Phe Thr Met Ala Gly Thr Val Trp Trp Val Ile Leu Thr
 290 295 300
 Ile Thr Trp Phe Leu Ala Ala Gly Arg Lys Trp Ser Cys Glu Ala Ile
 305 310 315 320
 Glu Gln Lys Ala Val Trp Phe His Ala Val Ala Trp Gly Ala Pro Gly
 325 330 335
 Phe Leu Thr Val Met Leu Leu Ala Met Asn Lys Val Glu Gly Asp Asn
 340 345 350
 Ile Ser Gly Val Cys Phe Val Gly Leu Tyr Asp Leu Asp Ala Ser Arg

[illegible]

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2259 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: Mus musculus transmembrane receptor (frizzled 7) mRNA, Coding region: 362..2080

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTGAAGGTA ACCGGAGAAG CTTGTTGCTC GTCGCCGCAG AGAAAGCCGC ACCGTTACGT	60
CTCGGGGGGA GGGTAAGGCG ACACCCCTTC CCTCGTACCC CCACTCCAGG CCCAGGAGTT	120
TGAACTCCGG CGGCTGCGTG AGTGCCACGT GGAGGCGGCT GCGGCGCCCC TCGGCTGGCG	180
GCCTCGCCCC CGCTGTGCAG GCACCCTAGC ACCCTCGGCT CCGCGCCGCC CACGGCGGCC	240
CCGGCGCCGG GAGGACTCTC ATGCGCCGGC CGGGCGGCGG CGCCTCCCTG TATCCAAGCC	300
TCTCCCCAGC GCCTCGTCTT TTCTCTCCAG CTGAGAACGC CGCTGCACTC GCGACCGGCG	360
ATGCGGGGCC CCGGCACGGC GGCCTCGCAC TCGCCCCTGG GCCTCTGCGC CCTGGTGCTT	420
GCTCTTCTGG GCGCGCTGCC CACGGACACC CGGGCTCAGC CATATCACGG CGAGAAAGGC	480
ATCTCGGTAC CGGACCACGG CTTCTGCCAG CCCATCTCCA TCCCGTTGTG CACGGATATC	540
GCCTACAACC AGACCATCCT GCCCAACCTG CTGGGCCACA CGAACCAAGA GGACGCGGGC	600
CTCGAGGTGC ACCAGTTCTA CCCTCTGGTA AAGGTGCAGT GTTCTCCTGA GCTACGCTTC	660
TTCTTATGCT CTATGTACGC ACCCGTGTGC ACCGTGCTCG ACCAAGCCAT TCCTCCGTGC	720
CGTTCCTTGT GCGAGCGCGC CCGACAGGGC TCGAGGCGC TCATGAACAA GTTCGGCTTC	780
CAGTGGCCAG AGCGGTTGCG CTGCGAGAAC TTCCAGTGC ACGGTGCCGG CGAGATCTGC	840
GTGGGGCAGA ACACGTCCGA CGGCTCCGGG GGCGCGGGCG GCAGTCCCAC CGCCTACCCT	900
ACTGCTCCCT ACCTGCCAGA CCCACCTTTC ACTGCGATGT CCCCCTCAGA TGGCAGAGGC	960
CGTTTGCTT TCCCTTCTC GTGTCCGCGC CAGCTCAAGG TGCCCCCTA CCTGGGCTAC	1020
CGTTTCCTAG GTGAGCGTGA CTGCGGTGCC CCGTGTGAGC CGGGCCGTGC TAACGGCCTC	1080
ATGTACTTTA AAGAAGAGGA GAGACGGTTC GCCCGCCTCT GGGTGGGTGT GTGGTCAGTG	1140
CTGTCGTGCG CCTCGACGCT CTTACGGTG CTCACCTACC TAGTGGACAT GCGTCGCTTC	1200
AGCTATCCAG AGCGACCCAT CATCTTCCTG TCGGGTTGCT ACTTCATGGT GGCAGTGGCG	1260
CACGTGGCAG GCTTCCTGCT AGAGGACCGT GCCGTGTGCG TGGAGCGCTT CTCGGACGAT	1320
GGCTACCGCA CGGTGGCGCA GGGCACCAAG AAGGAGGGCT GCACCATCCT CTTTCATGGTG	1380

```

CTTTACTTCT TCGGTATGGC CAGCTCCATC TGGTGGGTCA TTCTGTCCCT CACTTGGTTC      1440
CTGGCAGCTG GCATGAAGTG GGGCCACGAG GCCATCGAGG CCAACTCGCA GTACTTTTCAT      1500
CTGGCCGCGT GGGCTGTGCC AGCGGTCAAG ACAATCACCA TTTTGGCCAT GGGCCAGGTG      1560
GATGGTGACC TACTCAGTGG AGTGTGCTAC GTGGGCCTGT CTAGTGTGGA TGCATTGCGG      1620
GGCTTCGTGC TGGCGCCCTT GTTCGTCTAC CTCTTCATCG GGACGTCCTT CCTGTTGGCC      1680
GGCTTTGTGT CTCTCTTTTCG CATCCGCACC ATCATGAAGC ACGACGGCAC CAAGACAGAG      1740
AAGCTGGAGA AGCTGATGGT GCGCATCGGC GTCTTCAGCG TGCTCTACAC GGTGCCGGCC      1800
ACCATCGTGT TGGCCTGCTA CTTTTATGAG CAGGCCTTCC GAGAGCACTG GGAACGCACC      1860
TGGCTCCTGC AGACTTGCAA GAGCTACGCT GTGCCCTGCC CTCCGCGCCA CTTCTCTCCC      1920
ATGAGCCCCG ACTTTACAGT CTTTCATGATC AAGTACCTGA TGACCATGAT CGTGGGCATC      1980
ACTACGGGCT TCTGGATCTG GTCGGGCAAG ACCCTGCAGT CATGGCGTCG CTTCTACCAC      2040
AGACTCAGCC ACAGCAGCAA GGGGGAAACT GCGGTATGAG CCCCGGTCCT TACCCACCCT      2100
TGCCTCTTCT ACCCTTTTAC AGGAGGAGAG GCATGGTAGG GAGAGAACTG CTGGGTGGGG      2160
GCTTGTTTCC GTAAGCTACC TGCCCCCTCC ACTGAGCTTT AACCTGGAAG TGAGAAGTTA      2220
TTTGGAGGTG AGAAGAGATT TGGGGGCGAG AGATGGTTT      2259

```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 573 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Mfz7 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

Met Arg Gly Pro Gly Thr Ala Ala Ser His Ser Pro Leu Gly Leu Cys
1           5           10           15
Ala Leu Val Leu Ala Leu Leu Gly Ala Leu Pro Thr Asp Thr Arg Ala
20           25           30
Gln Pro Tyr His Gly Glu Lys Gly Ile Ser Val Pro Asp His Gly Phe
35           40           45
Cys Gln Pro Ile Ser Ile Pro Leu Cys Thr Asp Ile Ala Tyr Asn Gln
50           55           60
Thr Ile Leu Pro Asn Leu Leu Gly His Thr Asn Gln Glu Asp Ala Gly
65           70           75           80
Leu Glu Val His Gln Phe Tyr Pro Leu Val Lys Val Gln Cys Ser Pro
85           90           95

```

45

Glu Leu Arg Phe Phe Leu Cys Ser Met Tyr Ala Pro Val Cys Thr Val
 100 105 110
 Leu Asp Gln Ala Ile Pro Pro Cys Arg Ser Leu Cys Glu Arg Ala Arg
 115 120 125
 Gln Gly Cys Glu Ala Leu Met Asn Lys Phe Gly Phe Gln Trp Pro Glu
 130 135 140
 Arg Leu Arg Cys Glu Asn Phe Pro Val His Gly Ala Gly Glu Ile Cys
 145 150 155 160
 Val Gly Gln Asn Thr Ser Asp Gly Ser Gly Gly Ala Gly Gly Ser Pro
 165 170 175
 Thr Ala Tyr Pro Thr Ala Pro Tyr Leu Pro Asp Pro Pro Phe Thr Ala
 180 185 190
 Met Ser Pro Ser Asp Gly Arg Gly Arg Leu Ser Phe Pro Phe Ser Cys
 195 200 205
 Pro Arg Gln Leu Lys Val Pro Pro Tyr Leu Gly Tyr Arg Phe Leu Gly
 210 215 220
 Glu Arg Asp Cys Gly Ala Pro Cys Glu Pro Gly Arg Ala Asn Gly Leu
 225 230 235 240
 Met Tyr Phe Lys Glu Glu Glu Arg Arg Phe Ala Arg Leu Trp Val Gly
 245 250 255
 Val Trp Ser Val Leu Ser Cys Ala Ser Thr Leu Phe Thr Val Leu Thr
 260 265 270
 Tyr Leu Val Asp Met Arg Arg Phe Ser Tyr Pro Glu Arg Pro Ile Ile
 275 280 285
 Phe Leu Ser Gly Cys Tyr Phe Met Val Ala Val Ala His Val Ala Gly
 290 295 300
 Phe Leu Leu Glu Asp Arg Ala Val Cys Val Glu Arg Phe Ser Asp Asp
 305 310 315 320
 Gly Tyr Arg Thr Val Ala Gln Gly Thr Lys Lys Glu Gly Cys Thr Ile
 325 330 335
 Leu Phe Met Val Leu Tyr Phe Phe Gly Met Ala Ser Ser Ile Trp Trp
 340 345 350
 Val Ile Leu Ser Leu Thr Trp Phe Leu Ala Ala Gly Met Lys Trp Gly
 355 360 365
 His Glu Ala Ile Glu Ala Asn Ser Gln Tyr Phe His Leu Ala Ala Trp
 370 375 380
 Ala Val Pro Ala Val Lys Thr Ile Thr Ile Leu Ala Met Gly Gln Val
 385 390 395 400
 Asp Gly Asp Leu Leu Ser Gly Val Cys Tyr Val Gly Leu Ser Ser Val
 405 410 415
 Asp Ala Leu Arg Gly Phe Val Leu Ala Pro Leu Phe Val Tyr Leu Phe
 420 425 430
 Ile Gly Thr Ser Phe Leu Leu Ala Gly Phe Val Ser Leu Phe Arg Ile
 435 440 445
 Arg Thr Ile Met Lys His Asp Gly Thr Lys Thr Glu Lys Leu Glu Lys

450	455	460
Leu Met Val Arg Ile Gly Val Phe Ser Val Leu Tyr Thr Val Pro Ala		
465	470	475
Thr Ile Val Leu Ala Cys Tyr Phe Tyr Glu Gln Ala Phe Arg Glu His		
	485	490
Trp Glu Arg Thr Trp Leu Leu Gln Thr Cys Lys Ser Tyr Ala Val Pro		
	500	505
Cys Pro Pro Arg His Phe Ser Pro Met Ser Pro Asp Phe Thr Val Phe		
	515	520
Met Ile Lys Tyr Leu Met Thr Met Ile Val Gly Ile Thr Thr Gly Phe		
	530	535
Trp Ile Trp Ser Gly Lys Thr Leu Gln Ser Trp Arg Arg Phe Tyr His		
	545	550
Arg Leu Ser His Ser Ser Lys Gly Glu Thr Ala Val Glx		
	565	570

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2421 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: Mus musculus transmembrane receptor (frizzled 8) gene, Coding region: 188..2245

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGGGGAGGGC CGGACGACTC CAGCCTAGGT TTCCAACCCT GCTGCCTGAA AAGGAGATAG	60
ACTGTTGCTA TTCTCCTCTG CAGAGAAAAG TGGGACACGA CCCGCTCTCC CTTTCTCTCAG	120
ATTCCTCACT GCAGAGCCCT CCTGCGCGCC GCCTAGAGAA GGAGGACTTG GGGTCCCAGC	180
GCGCAGCATG GAGTGGGGTT ACCTGTTGGA AGTGACCTCG CTCCTAGCCG CTTTGGCGGT	240
GCTACAGCGC TCTAGCGGCG CTGCCGCGGC TTCGGCCAAG GAGCTGGCGT GCCAAGAGAT	300
CACGGTGCCG TTGTGCAAAG GCATCGGTTA CAACTACACT TACATGCCCA ACCAGTTCAA	360
CCACGACACG CAAGATGAGG CGGGCCTAGA GGTGCACCAG TTTTGGCCGC TGGTGGAGAT	420
ACAGTGCTCC CCGGACCTCA AGTTCTTTCT GTGTAGCATG TACACGCCCA TCTGCCTGGA	480
GGACTACAAG AAGCCTCTGC CGCCTTGTCG CTCTGTGTGT GAACGCGCCA AGGCCGGCTG	540
CGCGCCGCTC ATGCGCCAGT ACGGCTTTGC TTGGCCTGAC CGCATGCGCT GCGATCGGTT	600
GCCGGAGCAG GGCAACCCGG ACACTCTGTG CATGGACTAC AACC GCACCG ACCTCACCAC	660
GGCCGCGCCC AGCCACCGC GCCGCCTGCC TCCGCCGCCT CCTCCCGGCG AGCAGCCGCC	720

CTCTGGCAGC	GGCCACAGCC	GCCCCGCCAGG	GGCCAGGCCC	CCACATCGTG	GCGGCAGCAG	780
TAGGGGCAGC	GGGGACGCGG	CGGCTGCGCC	CCCTTCGCGC	GGCGGGAAGG	CGAGGCCCCC	840
TGGTGGCGGC	GCTGCTCCCT	GCGAGCCGGG	GTGCCAGTGC	CGCGCGCCCA	TGGTGAGCGT	900
GTCCAGCGAA	CGCCACCCGC	TCTACAACCG	CGTCAAGACC	GGCCAGATCG	CCAAGTGTGC	960
GCTGCCCTGC	CACAACCCCT	TCTTTAGCCA	GGATGAGCGC	GCCTTCACCG	TCTTCTGGAT	1020
CGGCCTGTGG	TCGGTGCTCT	GCTTCGTCTC	CACCTTCGCC	ACTGTCTCTA	CCTTCCTCAT	1080
CGATATGGAG	CGCTTTAAGT	ACCCGGAACG	GCCCATCATA	TTCCTCTCCG	CCTGTTACCT	1140
CTTCGTGTCT	GTCGGGTACC	TGGTGCGCCT	GGTGGCAGGA	CATGAGAAAG	TGGCCTGCAG	1200
CGGCGGCGCT	CCGGGTGCTG	GCGGACGTGG	GGGTGCGGGC	GGCGCGGCGG	CGGCTGGCGC	1260
AGGGGCAGCG	GGACGGGGGG	CGAGCAGCCC	GGGCGCGCGC	GGCGAGTACG	AGGAGCTGGG	1320
CGCAGTTGAG	CAGCATGTTC	GCTATGAGAC	CACTGGCCCC	GCGCTGTGCA	CGGTGGTCTT	1380
TCTCCTTGTC	TACTTTTTTG	GCATGGCCAG	CTCCATCTGG	TGGGTAATCC	TGTCGCTCAC	1440
GTGGTTCTTG	GCAGCTGGCA	TGAAGTGGGG	TAACGAGGCC	ATAGCAGGCT	ACTCGCAGTA	1500
CTTCCACCTG	GCCGCGTGCC	TTGTGCCCAG	CGTCAAGTCC	ATCGCGGTGC	TGGCGCTCAG	1560
CTCCGTAGAC	GGCGACCCGG	TGGCGGGCAT	CTGCTACGTG	GGCAACCAGA	GCCTTGACAA	1620
CCTACGCGGC	TTTGTGCTGG	CGCCACTGGT	TATCTACCTC	TTCATTGGGA	CTATGTTTCT	1680
GTTAGCTGGC	TTCGTGTGCG	TGTTCCGAAT	CCGTTCAGTC	ATCAAGCAGC	AAGGAGGTCC	1740
AACTAAGACA	CACAAGCTAG	AAAAACTCAT	GATCCGCTTG	GGCCTCTTCA	CCGTGCTCTA	1800
CACGGTGCCC	GCTGCCGTCG	TTGTGCGCCT	CCTTTTCTAT	GAGCAGCACA	ACCGACCGCG	1860
CTGGGAGGCC	ACGCACAACCT	GCCCATGCCT	TCGGGACCTG	CAACCGGACC	AGGCTCGCAG	1920
GCCCGATTAC	GCGGTCTTCA	TGCTCAAGTA	CTTCATGTGC	CTAGTAGTGG	GCATCACATC	1980
GGGCGTGTGG	GTCTGGTCCG	GCAAGACTCT	GGAGTCCTGG	CGCGCGTTGT	GCACTAGGTG	2040
CTGCTGGGCC	AGCAAGGGCG	CTGCAGTAGG	CGCGGGCGCT	GGAGGCAGCG	GCCCTGGGGG	2100
CAGTGGAACC	GGGCCCCGGC	GAGGTGGGGG	ACACGGCGGA	GGCGGGGGAT	CCCTCTACAG	2160
CGACGTCACT	ACCGGCCTGA	CGTGGCGGTC	TGGCACGGCC	AGCTCTGTAT	CTTACCCTAA	2220
GCAAATGCCA	TTGTCCCAGG	TCTGAACCCCT	ACGTGGATGC	CCAGAAGGGG	CGGAGAGGAG	2280
TGGGGGATGG	GGAACCCGTG	GGCGGCGAAG	GGACCCCAGA	CCGGCCAGGG	TTCCCACCCC	2340
TTCCCAGTGT	TGACTGCTAT	AGCATGACAA	TGAAGTGTTA	ATGGTATCCA	TTAGCAGCGG	2400
GGACTTAAAT	GACTCCCTTA	G				2421

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 682 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Mfz8 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

Met Glu Trp Gly Tyr Leu Leu Glu Val Thr Ser Leu Leu Ala Ala Leu
 1          5          10          15
Ala Val Leu Gln Arg Ser Ser Gly Ala Ala Ala Ser Ala Lys Glu
          20          25          30
Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys Lys Gly Ile Gly Tyr
          35          40          45
Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His Asp Thr Gln Asp Glu
          50          55          60
Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Gln Cys
          65          70          75          80
Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met Tyr Thr Pro Ile Cys
          85          90          95
Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys Arg Ser Val Cys Glu
          100          105          110
Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg Gln Tyr Gly Phe Ala
          115          120          125
Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro Glu Gln Gly Asn Pro
          130          135          140
Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp Leu Thr Thr Ala Ala
          145          150          155          160
Pro Ser Pro Pro Arg Arg Leu Pro Pro Pro Pro Pro Gly Glu Gln
          165          170          175
Pro Pro Ser Gly Ser Gly His Ser Arg Pro Pro Gly Ala Arg Pro Pro
          180          185          190
His Arg Gly Gly Ser Ser Arg Gly Ser Gly Asp Ala Ala Ala Ala Pro
          195          200          205
Pro Ser Arg Gly Gly Lys Ala Arg Pro Pro Gly Gly Gly Ala Ala Pro
          210          215          220
Cys Glu Pro Gly Cys Gln Cys Arg Ala Pro Met Val Ser Val Ser Ser
          225          230          235          240
Glu Arg His Pro Leu Tyr Asn Arg Val Lys Thr Gly Gln Ile Ala Asn
          245          250          255
Cys Ala Leu Pro Cys His Asn Pro Phe Phe Ser Gln Asp Glu Arg Ala
          260          265          270
Phe Thr Val Phe Trp Ile Gly Leu Trp Ser Val Leu Cys Phe Val Ser
          275          280          285
Thr Phe Ala Thr Val Ser Thr Phe Leu Ile Asp Met Glu Arg Phe Lys
          290          295          300
Tyr Pro Glu Arg Pro Ile Ile Phe Leu Ser Ala Cys Tyr Leu Phe Val

```

305					310						315				320
Ser	Val	Gly	Tyr	Leu	Val	Arg	Leu	Val	Ala	Gly	His	Glu	Lys	Val	Ala
				325					330					335	
Cys	Ser	Gly	Gly	Ala	Pro	Gly	Ala	Gly	Gly	Arg	Gly	Gly	Ala	Gly	Gly
			340					345					350		
Ala	Ala	Ala	Ala	Gly	Ala	Gly	Ala	Ala	Gly	Arg	Gly	Ala	Ser	Ser	Pro
			355				360					365			
Gly	Ala	Arg	Gly	Glu	Tyr	Glu	Glu	Leu	Gly	Ala	Val	Glu	Gln	His	Val
	370					375					380				
Arg	Tyr	Glu	Thr	Thr	Gly	Pro	Ala	Leu	Cys	Thr	Val	Val	Phe	Leu	Leu
385					390					395					400
Val	Tyr	Phe	Phe	Gly	Met	Ala	Ser	Ser	Ile	Trp	Trp	Val	Ile	Leu	Ser
				405					410					415	
Leu	Thr	Trp	Phe	Leu	Ala	Ala	Gly	Met	Lys	Trp	Gly	Asn	Glu	Ala	Ile
			420					425					430		
Ala	Gly	Tyr	Ser	Gln	Tyr	Phe	His	Leu	Ala	Ala	Trp	Leu	Val	Pro	Ser
		435					440					445			
Val	Lys	Ser	Ile	Ala	Val	Leu	Ala	Leu	Ser	Ser	Val	Asp	Gly	Asp	Pro
	450					455					460				
Val	Ala	Gly	Ile	Cys	Tyr	Val	Gly	Asn	Gln	Ser	Leu	Asp	Asn	Leu	Arg
465					470				475						480
Gly	Phe	Val	Leu	Ala	Pro	Leu	Val	Ile	Tyr	Leu	Phe	Ile	Gly	Thr	Met
				485					490					495	
Phe	Leu	Leu	Ala	Gly	Phe	Val	Ser	Leu	Phe	Arg	Ile	Arg	Ser	Val	Ile
			500					505					510		
Lys	Gln	Gln	Gly	Gly	Pro	Thr	Lys	Thr	His	Lys	Leu	Glu	Lys	Leu	Met
		515					520					525			
Ile	Arg	Leu	Gly	Leu	Phe	Thr	Val	Leu	Tyr	Thr	Val	Pro	Ala	Ala	Val
	530					535					540				
Val	Val	Ala	Cys	Leu	Phe	Tyr	Glu	Gln	His	Asn	Arg	Pro	Arg	Trp	Glu
545					550					555					560
Ala	Thr	His	Asn	Cys	Pro	Cys	Leu	Arg	Asp	Leu	Gln	Pro	Asp	Gln	Ala
				565					570					575	
Arg	Arg	Pro	Asp	Tyr	Ala	Val	Phe	Met	Leu	Lys	Tyr	Phe	Met	Cys	Leu
			580					585					590		
Val	Val	Gly	Ile	Thr	Ser	Gly	Val	Trp	Val	Trp	Ser	Gly	Lys	Thr	Leu
		595					600					605			
Glu	Ser	Trp	Arg	Ala	Leu	Cys	Thr	Arg	Cys	Cys	Trp	Ala	Ser	Lys	Gly
	610					615					620				
Ala	Ala	Val	Gly	Ala	Gly	Ala	Gly	Gly	Ser	Gly	Pro	Gly	Gly	Ser	Gly
625					630					635					640
Pro	Gly	Pro	Gly	Gly	Gly	Gly	Gly	His	Gly	Gly	Gly	Gly	Gly	Ser	Leu
				645					650					655	
Tyr	Ser	Asp	Val	Ser	Thr	Gly	Leu	Thr	Trp	Arg	Ser	Gly	Thr	Ala	Ser
			660					665					670		

Ser Val Ser Tyr Pro Lys Gln Met Pro Leu
675 680

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: Amino acid sequence used to design YW157 sense primer
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Tyr Pro Glu Arg Pro Ile
1 5

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: Amino acid sequence used to design YW158 antisense primer
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Trp Phe Leu Ala Ala
1 5

IT IS CLAIMED:

1. A method of identifying a compound capable of affecting binding of a Wnt polypeptide to a Wnt receptor (WntR) polypeptide, comprising
5 contacting such a WntR polypeptide with a selected Wnt polypeptide, in the presence and absence of a test compound,
measuring the effect of the test compound on the extent of binding between said Wnt and said WntR, and
identifying said compound as effective to alter binding of a Wnt polypeptide to a
10 WntR polypeptide if its measured effect on the extent of binding is above a threshold level.
2. The method of claim 1, wherein said threshold is a 2-fold or greater inhibition of binding.
- 15 3. The method of claim 1, wherein said threshold is a 2-fold or greater potentiation of binding.
4. The method of claim 1, wherein said Wnt polypeptide is *wingless* (Wg).
- 20 5. The method of claim 1, wherein said WntR polypeptide is Dfz2.
6. The method of claim 5, wherein said WntR polypeptide has the amino acid sequence represented as SEQ ID NO:2.
- 25 7. The method of claim 1, wherein said test compound is effective to inhibit binding between the Wnt polypeptide and the WntR polypeptide.
8. The method of claim 1, wherein said test compound is effective to displace the Wnt polypeptide from the WntR polypeptide.
- 30 9. The method of claim 1, wherein said WntR polypeptide is expressed on the surface of a cell transformed with an expression vector encoding said receptor.

10. The method of claim 9, wherein said cell is a *Drosophila* Schneider 2 (S2) cell and said expression vector encodes the WntR polypeptide Dfz2.

11. The method of claim 1, wherein said WntR polypeptide is an N-terminal
5 portion of a full-length WntR polypeptide, said portion including the cysteine-rich amino-terminal domain.

12. The method of claim 11, wherein said portion is a first part of a fusion protein.

10 13. The method of claim 12, wherein said fusion protein further includes a second portion, said second portion containing the constant domain of human IgG.

14. The method of claim 1, further comprising preparing a pharmaceutical
preparation of a compound identified as effective to alter binding of a Wnt polypeptide to a
15 WntR polypeptide.

1/6

Dfz2	MRHNRLKVL	LGLVLLLTSC	RADGPLHSAD	HGMGGMGMGG	HGLDASPAPG	50
Dfz1	MWRQILFILP	-TLIQGVQRY	-DQSPLDASP	YYRSGGGL--	--M---ASSG	41
Consensus	M....L..L.	..L.....PL....G.G...G	50
Dfz2	YGVPAIPKDP	NLRCEEITIP	MCRGIGYNMT	SFPNEMNHET	QDEAGLEVHQ	100
Dfz1	TELDGLPHHN	--RCEPITIS	ICKNIPYNMT	IMPNLIGHTK	QEEAGLEVHQ	89
ConsensusP...	..RCE.ITI.	.C..I.YNMT	..PN...H..	Q.EAGLEVHQ	100
Dfz2	FWPLVEIKCS	PDLKFFLCSM	YTPICLEDYH	KPLPVCRSVC	ERARSGCAPI	150
Dfz1	FAPLVKIGCS	DDLQLFLCSL	YVPVC-TILE	RPIPPCCSLC	ESAR-VCEKL	137
Consensus	F.PLV.I.CS	.DL..FLCS.	Y.P.C.....	.P.P.CRS.C	E.AR..C...	150
Dfz2	MQQYSFEWPE	RMACEHLPLH	GDPDNLCMEQ	PSYTEAGSGG	SSGSGSGSGS	200
Dfz1	MKTYNFWWPE	NLECSKFPVH	GGED-LCVAE	-----NTTS	SASTAATPTR	180
Consensus	M..Y.F.WPE	...C...P.H	G..D.LC...	S.....	200
Dfz2	GSGSGGKRKQ	GGSGSGGSGA	GGSSGSTSTK	PCRGRNSKNC	QNPQGEKASG	250
Dfz1	SVAKVTTRKH	-----QTGV	-----	-----	ESPH--RNIG	202
ConsensusRK.G.P.....G	250
Dfz2	KECSCSCRSP	LIFLGKEQLL	QQQSQMPMMH	HPHHWYMNLT	VQRIAGVPNC	300
Dfz1	FVC-----P	V-----QL-	--KTPLGMG-	-----Y-ELK	VG-GKDLHDC	229
Consensus	..C.....PQL.M..Y..L.	V.....C	300
Dfz2	GIPCKGPFFS	NDEKDFAGLW	IALWSGLCFC	STLMTLTTFI	IDTERFKYPE	350
Dfz1	GAPCHAMFFP	ERERTVLRVW	VGSWAAVCVA	SCLFTVLTFI	IDSSRFYYPE	279
Consensus	G.PC...FF.	..E.....W	...W...C..	S.L.T..TF.	ID..RF.YPE	350
Dfz2	RPIVFLSACY	FMVAVGYLS-	-----R	N-FLQNEEIA	CDGLL--LRE	387
Dfz1	RAIVFLAVCY	LVVGCAYVAG	LGAGDSVSCR	EPFPPPVKLG	RLQMMSTITQ	329
Consensus	R.IVFL..CY	..V...Y...R	..F.....	400
Dfz2	SSTGPHSCTL	VFLLTYYFF-G	MASSIWWVIL	SFTWFLAAGL	KWGNEAITKH	436
Dfz1	GHRQTTSTCTV	LFM-ALYFCC	MAFAWWSCL	AFWFLAAGL	KWGHEAIENK	378
ConsensusSCT.	.F.....F..	MA...WW..L	.F.WFLAAGL	KWG.EAI...	450
Dfz2	SQYFHLAAWL	IPTVQSVAVL	LLSAVDGDPI	LGICYVGNLN	PDHLKTFVLA	486
Dfz1	SHLFLVAVAW	VPALQTISVL	ALAKVEGDIL	SGVCFVGQLD	THSLGAFLIL	428
Consensus	S..FHL.AW.	.P..Q...VL	.L..V.GD..	.G.C.VG.L.	...L..F...	500
Dfz2	PLFVYLVIGT	TFLMAGFVSL	FRIRSVIKQQ	GGVGAGVKAD	KLEKLMIRIG	536
Dfz1	PLCIYLSIGA	LFLLAGFISL	FRIRTVMKTD	G-----KRTD	KLERLMLRIG	473
Consensus	PL..YL.IG.	.FL.AGF.SL	FRIR.V.K..	G.....D	KLE.LM.RIG	550
Dfz2	IFSVLYTVPA	TIVIGCYLYE	AAYFEDWI--	-----KALA	CPCAQVKGPG	578
Dfz1	FFSGLFILPA	VGLLGCLFYE	YYNFDEWMIQ	WHRDICKPFS	IPCFAARAPG	523
Consensus	.FS.L...PAGC..YE	...F..W...K...	.PC.....PG	600
Dfz2	K---KPLYSV	LMLKYFMALA	VGITSGVWIW	SGKTLESWRR	FWRRLLGAPD	625
Dfz1	SPEARPIFQI	FMVKYLCMSL	VGVTSSVWLY	SSKTMVSWRN	FVERLQKKEP	573
ConsensusP....	.M.KY.....	VG.TS.VW..	S.KT..SWR.	F..RL.G...	650
Dfz2	RTGANQALIK	QRPPIPHPYA	GSGMGMPVGS	AAGSLLATPY	TQAGGASVAS	675
Dfz1	RT-----	-R---AQAYV	-----	-----	-----	581
Consensus	RT.....	.R.....Y.	700
Dfz2	TSHHHLHHHV	LKQPAASHV	694			
Dfz1	-----	-----	581			
Consensus	719			

Fig. 1

2/6

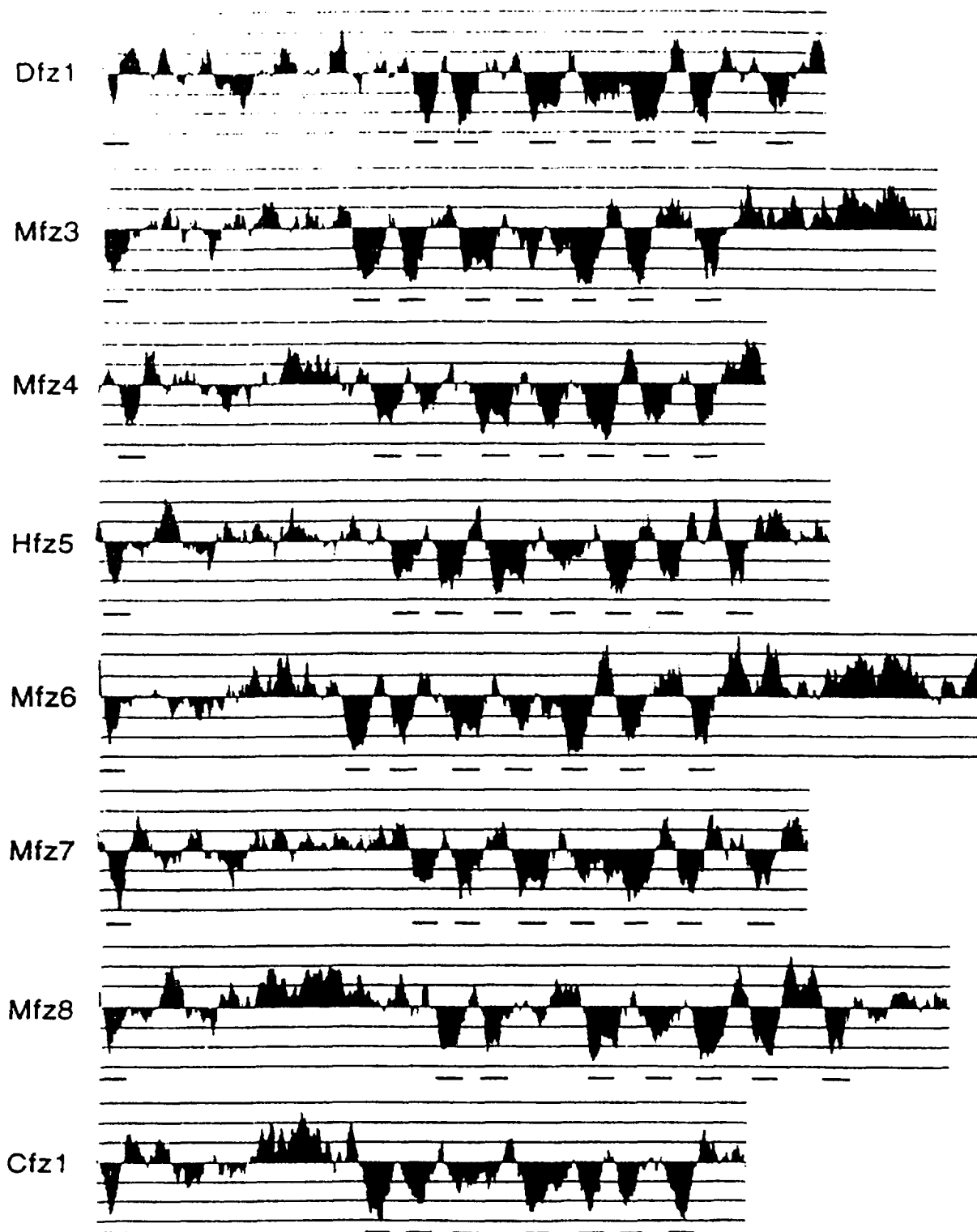
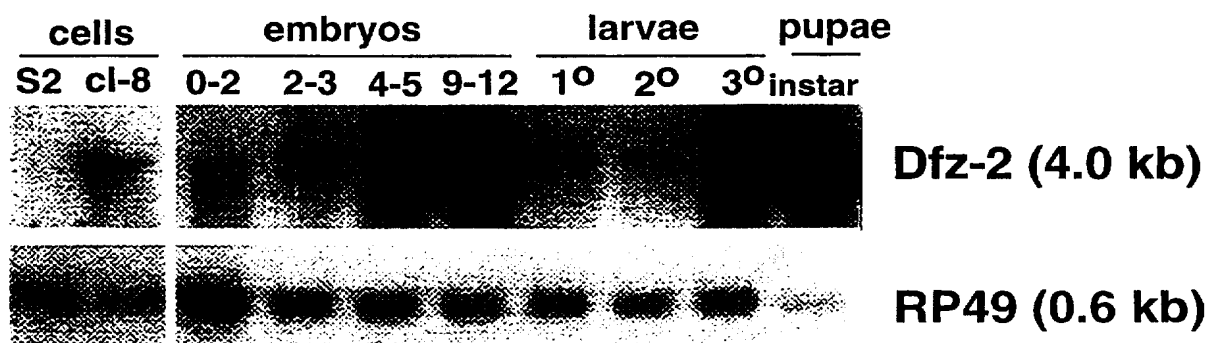
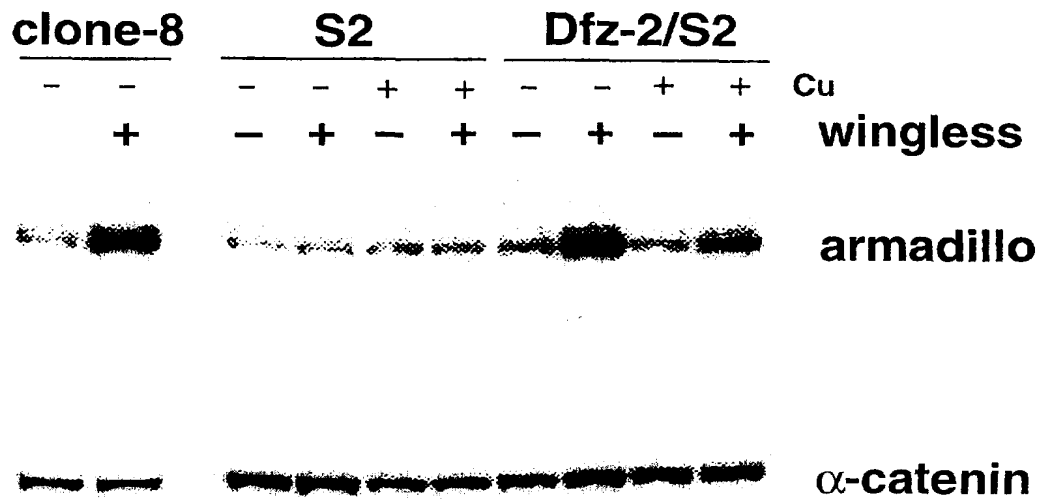


Fig. 2

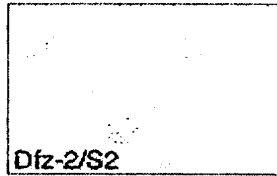
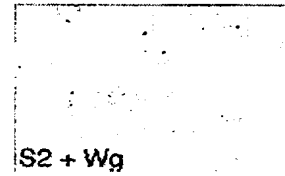
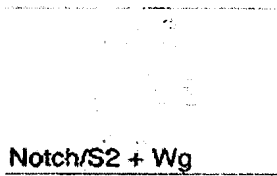
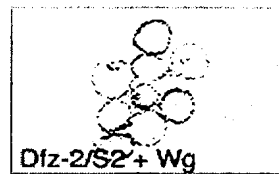
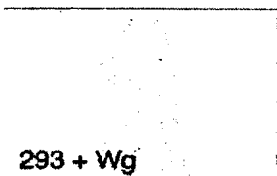
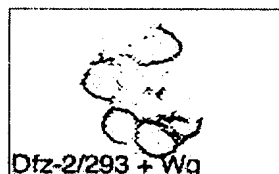
3/6

**Fig. 3**

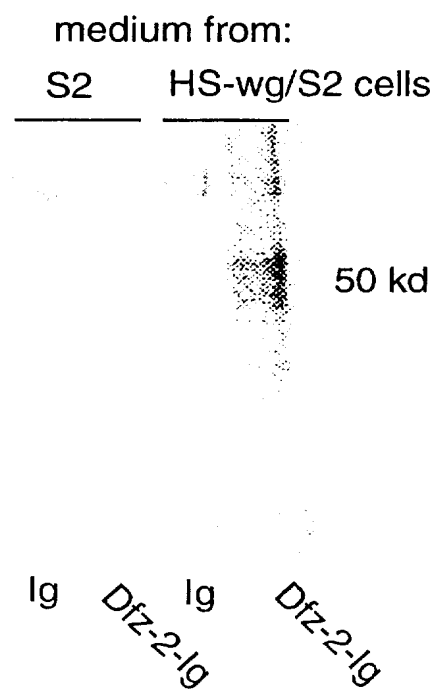
4/6

**Fig. 4**

5/6

**Fig. 5A****Fig. 5B****Fig. 5C****Fig. 5D****Fig. 5E****Fig. 5F**

6/6

**Fig. 6**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06049

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 33/566; C12N 15/12; A61K 38/19; C07K 14/705

US CL : 435/7.2, 69.1, 69.7; 424/85.1; 530/350, 351

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.2, 69.1, 69.7; 424/85.1; 530/350, 351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, CAPLUS, MEDLINE, APS, DIALOG, PIR50, A-GENESEQ26, SWISS-PROT34
search terms: Wnt, wingless, Dfz2, receptor, IgG, S2 cells

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 95/17416 A1 (MERCK & CO., INC.) 29 June 1995 (29.06.95), see page 11, lines 14-29 and EXAMPLE 8.	1, 7-9 and 14 ----- 2 and 3
A,P	US 5,585,087 A (LUSTIG et al.) 17 December 1996 (17.12.96).	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 03 JUNE 1997	Date of mailing of the international search report 08 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer DARYL A. BASHAM
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196